



# ARCHIVES OF PATHOLOGY

## EDITORIAL BOARD

LUDVIG HEKTOEN, Chicago, Chief Editor

GRANVILLE A BENNETT, Chicago

WILEY DAVIS FORBUS, Durham N C

S B WOLBACH Boston

GEORGE H WHIPPLE, Rochester, N Y

FRANK R MENNE, Portland, Ore

Volume 39  
1945

PUBLISHERS

AMERICAN MEDICAL ASSOCIATION

CHICAGO, ILL





# CONTENTS OF VOLUME 39

---

JANUARY 1945 NUMBER 1

	PAGE
Role of Schistosomiasis in the Etiology of Cancer of the Liver in the Chinese H Hartz, M D, Curaçao, Netherlands West Indies	1
Experimental Haplosporangium Infection L L Ashburn, M D, and C W Emmons, Ph D, Bethesda, Md	3
Pancreatic Necrosis in Electric Shock Major Alfred M Glazer, Medical Corps, Army of the United States	9
Correlation Between Chemical and Morphologic Alterations in Experimental Atheroscle- rosis O J Pollak, M D, Taunton, Mass	11
Attempts to Produce Cerebral Atherosclerosis O J Pollak, M D, Taunton, Mass	16
Melanoma of the Small Intestine Peter A Herbut M D, and Willis E Manges, M D, Philadelphia	22
Chemical Basis of Fever with Inflammation Valy Menkin, M D, Durham, N C	28
Classification of Tumors of the Kidney George L Fite, M D, Bethesda, Md	37
<b>Case Reports</b>	
Fatal Panniculitis Captain Nathan B Friedman, Medical Corps, Army of the United States	42
Two Cases of Congenital Web of a Bronchus James E Wallace, M D, Pittsburgh	47
<b>Laboratory Methods and Technical Notes</b>	
A New Hemoglobin Stain for Histologic Use A Slightly Modified Van Gieson Stain R C Dunn M D and E C Thompson, M T Bethesda, Md	49
<b>General Reviews</b>	
Arteriosclerosis (To Be Continued) W C Hueper, M D, New York	51
<b>Notes and News</b>	66
<b>Book Reviews</b>	66

FEBRUARY 1945 NUMBER 2

Congenital Glycogenic Tumors of the Heart Thomas M Batchelor, B S, and Mark E Maun, M D, Detroit	67
Primary Tumor of the Heart Reuben Straus, M D, and Reuben Merliss, M D, Los Angeles	74
Esophageal Carcinoma in British West Indian and Panamanian Negroes A Study of the Incidence, Etiologic Factors and Pathologic Anatomy in Fifty Cases Major Wray J Tomlinson and First Lieutenant Lester A Wilson Jr, Medical Corps, Army of the United States	79
Experimental Hypertension Its Production in Dogs by Intravenous Injection of Strepto- cocci George F Dick, M D, Chicago	81
Ossified Cartilage with Myeloid Fat Marrow in the Aortic Ring of a Rabbit W C Hueper, M D, New York	89
Effects of an Abortifacient Paste ("Ultra-Jel") Report of a Death from Its Use and of an Experimental Study of Its Effects on Rabbits and Rats R Straus, M D, Los Angeles, and N De Nosaquo, M D, Washington, D C	91

FEBRUARY—*Continued*

	PAGE
Adenomyoepithelioma (Cylindroma) of Palatal Mucous Glands William H. Bauer, M.D., D.D.S., and Robert A. Fox, M.D., St. Louis	96
Experimental Nephropathies I A Method of Producing Controlled Selective Injury of Renal Units by Means of Chemical Agents James P. Simonds, M.D., and Opal E. Hepler, M.D., Chicago	103
Case Reports	
Myelomalacia of the Cervical Portion of the Spinal Cord, Probably the Result of Roentgen Therapy Lewis D. Stevenson, M.D., and Robert E. Eckhardt, M.D., Ph.D., New York	109
Mediastinal Teratoma Lieutenant Colonel Sloan J. Wilson and Major Reuben Cares, Medical Corps, Army of the United States	113
General Reviews	
Arteriosclerosis (To Be Concluded) W. C. Hueper, M.D., New York	117
Notes and News	131
Correspondence	
Importance of Titles That Accurately Reflect the Subjects of the Articles Harrison S. Martland, M.D., Newark, N. J.	132

## MARCH 1945 NUMBER 3

Experimental Nephropathies II Renal Phosphatase After Poisoning with Mercury Bichloride, Uranyl Nitrate and Potassium Dichromate Opal E. Hepler, M.D., Helen Gurley, M.S., and James P. Simonds, M.D., Chicago	133
Ischiopagus Tripus Report of Two Cases Captain Hans G. Schlumberger, Medical Corps, Army of the United States, and J. Elmer Gotwals, M.D., Phoenixville, Pa.	142
Studies on the Pathogenesis of Glomerulonephritis I Production of Autoantibodies to Kidney in Experimental Animals Philip A. Cavelti, M.D., and Else Staehelin Cavelti, San Francisco	148
Cysts and Cystic Tumors of the Mediastinum T. C. Laipply, M.D., Cleveland	153
Some Endocrinologic Considerations of Canine Neoplastic Diseases R. M. Mulligan, M.D., Denver	162
A Theory of Transposition of the Arterial Trunks Based on the Phylogenetic and Ontogenetic Development of the Heart Major Maurice Lev, Medical Corps, Army of the United States, and Otto Saphir, M.D., Chicago	172
Case Reports	
Paraphysial Cyst of the Third Ventricle Leo D. Moss, M.D., Olean, N. Y.	184
General Reviews	
Arteriosclerosis (Concluded) W. C. Hueper, M.D., New York	187
Book Reviews	217
Books Received	220

## APRIL 1945 NUMBER 4

Morphologic Alterations of the Neuron Due to Tumor Invasion Karl Stern, M.D., and Guy L. Odom, M.D., Montreal, Canada	221
Morbid Anatomy of Typhus as Seen in a Recent Guatemalan Epidemic Observations on the Disease Major Alfred Golden, Medical Corps, Army of the United States	226

APRIL—*Continued*

	PAGE
Genetic Analysis of the Induction of Tumors by Methylcholanthrene VIII Two Mutations Arising in Mice Following Injection of Methylcholanthrene L C Strong, Ph D, New Haven, Conn	232
Changes in the Uterus After Eradication of Endometrial Adenocarcinoma by Radiotherapy, with Particular Reference to an Infarct-like Radionecrotic Plaque in the Lining John F Sheehan, M D, Herbert E Schmitz, M D, and Janet Towne, M D, Chicago	237
Healing of Wounds in the Skin of Mice Painted with 20-Methylcholanthrene Martin Silberberg, M D, and Ruth Silberberg, M D, St Louis	257
A Presacral Cyst Apparently Arising from the Neurenteric Canal in a Newborn Infant W W Brandes, M D, and John B Sutton, M D, New York	265
Coronary Arteriosclerosis and Myocardial Infarction as Studied by an Injection Technic J B Holyoke, M D, Rochester, Minn	268
Case Reports	
Periarteritis Nodosa Producing Aneurysm of the Renal Artery and Hypertension Peter A Herbut, M D, and Alison H Price, M D, Philadelphia	274
Histologic Diagnosis of Rabies Ernesto Herzog, M D, Concepcion, Chile	279
Notes and News	280
Books Received	280

## MAY 1945 NUMBER 5

Chronic Exudative and Indurative Pneumonia Due to Inhalation of Shellac Edwin F. Hirsch, M D, and Homer B Russell, M D, Chicago	281
Diffuse Meningeal Fibroblastoma of the Brain and Spinal Cord A Report of Three Cases Samuel R Haythorn, M D, William Shapera, M D, and Hubert C Stewart, M D, Pittsburgh	287
Morphologic Studies of Rats Deprived of Essential Amino Acids I Phenylalanine Mark E Maun, M D, William M Cahill, Ph D, and Ruth M Davis, B A, Detroit	294
Pathology of Anaphylaxis Due to Sulfonamide Drugs B Black-Schaffer, M D, Richmond, Va	301
Primary Systemic Amyloidosis Stuart Lindsay, M D, San Francisco, and William F Knorp, M D, San Mateo, Calif	315
Proliferation of Muscle Cells in the Myometrium of the Nonpregnant Uterus Philip H Hartz, M D, Curaçao, Netherlands West Indies	323
Periarteritis Nodosa-like Lesions in Rats Fed Thiouracil David Marine, M D, and Emil J Baumann, Ph D, New York	325
Case Reports	
Latent Primary Carcinoma of the Thyroid Gland Nathan Mitchell, M D, Brooklyn	331
Adrenal Cortical Adenoma of the Epididymis Ann Freeman, M D, Chicago	336
Bronchial Asthma and Tuberculosis Peter A Herbut, M D, Philadelphia	338
General Reviews	
Neoplastic Diseases of the Human Hypophysis John E Kraus, M D, Peoria, Ill	343
Book Reviews	350
Notes and News	350

## JUNE 1945 NUMBER 6

	PAGE
Poikilocytosis in Dairy Cattle J T Reid, M S , C F Huffman, Ph D , and C W Duncan, M S , East Lansing, Mich	351
Experimental Reproduction of Maduromycotic Lesions in Rabbits Douglas Symmers, M D , New York	358
Phialophora Jeanselmi Comb N from Mycetoma of the Hand C W Emmons, Ph D , Bethesda, Md	364
Acute Agranulocytosis A Microscopic Study of Ulceronecrotic Gingiva and of Bone Marrow in a Case Captain George J Anday, Medical Corps, Army of the United States, and Balint Orban, M D , D D S , Chicago	369
Aortic Abnormalities in Dogs Used for Experimental Purposes W C Hueper, M D , New York	375
Combined Effects of an Estrogen and an Anterior Hypophysial Extract on the Skeleton of the Growing Mouse Martin Silberberg, M D , and Ruth Silberberg, M D , St Louis	381
Case Reports	
Tularemia Report of a Laboratory Infection Fatal on the Fifth Day, with Early Pulmonary Involvement, Autopsy L L Ashburn, M D , Bethesda, Md , and Seward E Miller, M D , Baltimore	388
Books Received	392
General Reviews	
Intracranial Vascular Tumors and Malformations Harold H Noran, M D , Ph D , Minneapolis	393
Book Reviews	417
General Index	419

## ROLE OF SCHISTOSOMIASIS IN THE ETIOLOGY OF CANCER OF THE LIVER IN THE CHINESE

PHILIP H HARTZ, MD

Pathologist of the Public Health Service

CURAAO, NETHERLANDS WEST INDIES

Discussing the causes of liver cell cancer in a paper on primary neoplasms of the liver, Warvi<sup>1</sup> expressed the opinion that the increased incidence of this type of tumor in the Chinese appears to be connected with the frequent occurrence of cirrhosis secondary to schistosomiasis in this race. His opinion is probably based on reports of cases in which schistosomiasis, cirrhosis of the liver and liver cell cancer occurred in the same patient. However, before an etiologic relationship between schistosomiasis, on one side, and cirrhosis and liver cell cancer, on the other side, can be assumed, the occurrence of the two diseases last mentioned without schistosomiasis with the same frequency in Chinese must be excluded. This can be effected by the postmortem examination of a large number of Chinese who are living in countries or regions where schistosomiasis does not occur.

Such investigations have been carried out by several Dutch pathologists working in Java and Sumatra and who, incidentally, were the first to point to the increased incidence of liver cell cancer in the Chinese and the Javanese.<sup>2</sup> In Java and Sumatra autochthonous schistosomiasis and clonorchiosis do not occur,<sup>3</sup> and both islands, especially Java, have a large Chinese population, part of which has been living on the islands for many generations.

Bonne and others,<sup>4</sup> surveying 2,362 autopsies on Chinese men dying in Java and Sumatra, noted cirrhosis of the liver was present in 5.8 per cent, in 1.4 per cent liver cell cancer was the cause of death. In 32, probably all immigrants from China, infection with *Schistosoma*

(7 cases) and *Clonorchis* (25 cases) was noted. Real cirrhosis was absent in most cases, though, as the authors remarked, Laennec's cirrhosis does not protect against infection with *Schistosoma*.

In none of these cases was death caused by the helminthic infection and no liver cell cancer was found in this group. Of 44 persons with carcinoma coming to autopsy among Chinese in Batavia, 14, or 31 per cent, had primary cancer of the liver. In none of these was there schistosomiasis. The incidence of cirrhosis and primary cancer of the liver in the Javanese and the Malaysians, who are living under the same conditions and are certainly free from schistosomiasis, is even greater.<sup>5</sup> Tull,<sup>6</sup> working in Singapore, observed flukes in most of his patients, but this can be explained by the fact that among the Chinese population of Singapore there are many more immigrants from Southern China, where schistosomiasis is frequent.

According to Snapper,<sup>7</sup> cirrhosis of the liver is extremely frequent in North China, where schistosomiasis is not indigenous. In a small series of autopsies<sup>1</sup> in Peiping, primary liver cell cancer constituted 6.2 per cent of all cancers, in comparison with the West this is still a high incidence.

In Curaçao, in the Netherlands West Indies I found 5 cases of liver cell cancer in 33 autopsies on Chinese men during eight years. In none of these 33 cases was there clinical, gross or microscopic evidence of schistosomiasis. In the same period in 1,350 autopsies on Negroes and white

1 Warvi, W. N. Arch Path **37** 367, 1944

2 Smijders, E. P., and Straub, M. Geneesk tijdschr v Nederl-Indie **61** 625, 1921

3 Bonne, C. Am J Cancer **25** 811, 1935

4 Bonne, C., and others. Geneesk tijdschr v Nederl-Indie **71** 506, 1931

5 Bonne, C. Am J Cancer **30** 435, 1937, footnote 3

6 Tull, J. C. J Path & Bact **35** 557, 1932, cited by Warvi<sup>1</sup>

7 Snapper, I. Chinese Lessons to Western Medicine, New York, Interscience Publishers, Inc., 1941

people liver cell cancer was observed only in 3 and hepatic cancer of the bile duct type only in 1. One biopsy specimen from a tumor of the liver proved to be liver cell cancer. My observations parallel those of Strong and Pitts<sup>8</sup> but cannot be used as evidence for an etiologic rela-

---

<sup>8</sup> Strong, G. F., and Pitts, H. H. *Arch. Int. Med.* **46**: 105, 1930, cited by Snapper<sup>7</sup> and Warvi<sup>1</sup>.

tionship between schistosomiasis and liver cell cancer.

The only conclusion that I can draw from the foregoing figures is that I fully agree with Bonne<sup>5</sup> in his statement that schistosomiasis does not play a role in the etiology of liver cell cancer in the Chinese. "helminthic infections of the liver are out of the problem" (Bonne).

# EXPERIMENTAL HAPLOSPORANGIUM INFECTION

L L ASHBURN, M D

Surgeon, United States Public Health Service

AND

C W EMMONS, P H D

Principal Mycologist, United States Public Health Service

BETHESDA, MD

During the course of field studies of coccidioidomycosis, 151 strains of *Haplosporangium parvum* and 35 strains of *Coccidioides immitis* were isolated from the lungs of naturally infected wild rodents. In subsequent laboratory studies the relationship of *H. parvum* to coccidioidomycosis has been investigated. The strains of this fungus differed in such details as colony type, abundance of conidia and virulence in experimentally infected animals. White mice were inoculated with representative strains, and 2 of these, nos 1093 and 234, which produced many conidia and seemed to be relatively virulent, were selected for use in further studies. In an earlier report the production of lesions in white mice experimentally infected with this fungus was mentioned. It is the purpose of this paper to describe the experimentally produced lesions in more detail.

*Haplosporangium* exhibited a rather low degree of virulence, and early experiments were therefore directed toward an attempt to reproduce the mycosis in laboratory animals by whatever method showed most promise of success.

## PROCEDURES

Intraperitoneal introduction of spores was not successful in producing infections. Since the naturally occurring disease in wild mice was pulmonary, attempts were made to introduce spores directly into the lungs by forcing the animals to inhale dry spores, by injecting a suspension of spores through the thoracic wall into the lung, by intracardial injection and by dropping a suspension of spores into the nose. In the inoculation of mice the last-named method was the most successful. Mice anesthetized with ether were held ventral side up with the head slightly elevated, and the suspension of spores was dropped over the nostrils. If the proper degree of anesthesia was reached, this method was uniformly successful.

Repeated exposure seems to be an important factor in the development of many mycoses and undoubtedly occurs in the case of *Haplosporangium* infection in wild mice under natural field conditions. Most of the experimentally infected mice were therefore given repeated

intranasal inoculations in an attempt to produce progressive infection. The effect of reinoculation was investigated systematically in two series of mice consisting of 96 and 104 animals, respectively. In each series all animals were given a preliminary intranasal inoculation, the inoculum being kept as nearly uniform in size as possible. After an interval varying from one to six weeks a second inoculation was given to about 75 per cent of the mice. Animals were killed at suitable intervals to determine the extent of the resulting infection. A single intranasal inoculation was sufficient to produce characteristic pulmonary lesions. These were visible on postmortem examination as gray to yellowish, flat or slightly raised, irregularly limited areas, their location and extent depending probably on the amount of inoculum actually entering the lung and the route it followed.

## GROSS LESIONS PRODUCED

In many animals killed several weeks after inoculation, minute, yellowish white, discrete, slightly raised lesions were found. These apparently were lesions which developed around cells of the fungus after the resolution of the primary reaction which often followed the introduction of the suspension of spores into the lung. The histologic structure of these lesions will be described in a later section. Lesions of similar type and extent were found in animals receiving one and two inoculations, but in some cases the reinoculation apparently increased the severity and the extent of the infection. No correlation was found between the interval elapsing between the first and the second inoculation and differences in the type and the extent of lesions.

## PROTECTIVE EFFECT

The close association of *Haplosporangium* infection with pulmonary coccidioidomycosis in certain species of wild rodents in Arizona,<sup>1a</sup> the low virulence of *H. parvum* in experimentally infected white mice and the chronic nature of the infection produced in these animals suggested that it would be worth while to determine whether an initial infection with *H. parvum*

From the Pathology Laboratory and the Division of Infectious Diseases, National Institute of Health

1 (a) Emmons, C W, and Ashburn, L L. *Pub Health Rep* 57 1715-1727, 1942. (b) Ashburn, L L, and Emmons, C W. *Arch Path* 34 791-800, 1942.



would protect mice against subsequent exposure to *C. immitis*. In order to test this point, 33 white mice and white-footed mice (*Peromyscus leucopus*) were inoculated intranasally with *H. parvum* and after an interval of three weeks were inoculated intraperitoneally with an estimated dose of 11,000 spores of *C. immitis*. The strain of *C. immitis* used had been isolated from a pocket mouse (*Perognathus baileyi*) found spontaneously infected in Arizona. It as well as the other rodent strains of this fungus, had been compared with strains isolated from man and were found to be typical of the species in virulence and other characteristics. All but 2 of the *Peromyscus* mice died with typical coccidioidomycosis within two weeks after being inoculated with *C. immitis*. All but 6 of the white mice died from two to six weeks after that inoculation, the remaining 6 being put to death at that time. All showed lesions typical of coccidioidomycosis. As a control in this experiment, 19 mice were given the same dose of spores of *Coccidioides* without having had a preliminary infection with *Haplosporangium*. The progress of coccidioidomycosis in these control mice was the same as in the animals inoculated with both fungi. Similar results were obtained in a repetition of the experiment. There was no evidence that a previous exposure to *H. parvum* altered the resistance of mice experimentally infected with *C. immitis*.

#### LABORATORY ANIMALS USED

*H. parvum* produces in white mice pulmonary lesions which are like some of those found in naturally infected wild mice, but we have not yet succeeded in producing in these mice granulomas of the type found in some of the wild mice from which *H. parvum* only was isolated. The fungus, so far as present studies have shown, does not multiply within the body of the experimentally infected animal but undergoes a limited development, as described in the section on pathology. We have therefore attempted to find a laboratory animal more susceptible than the white mouse.

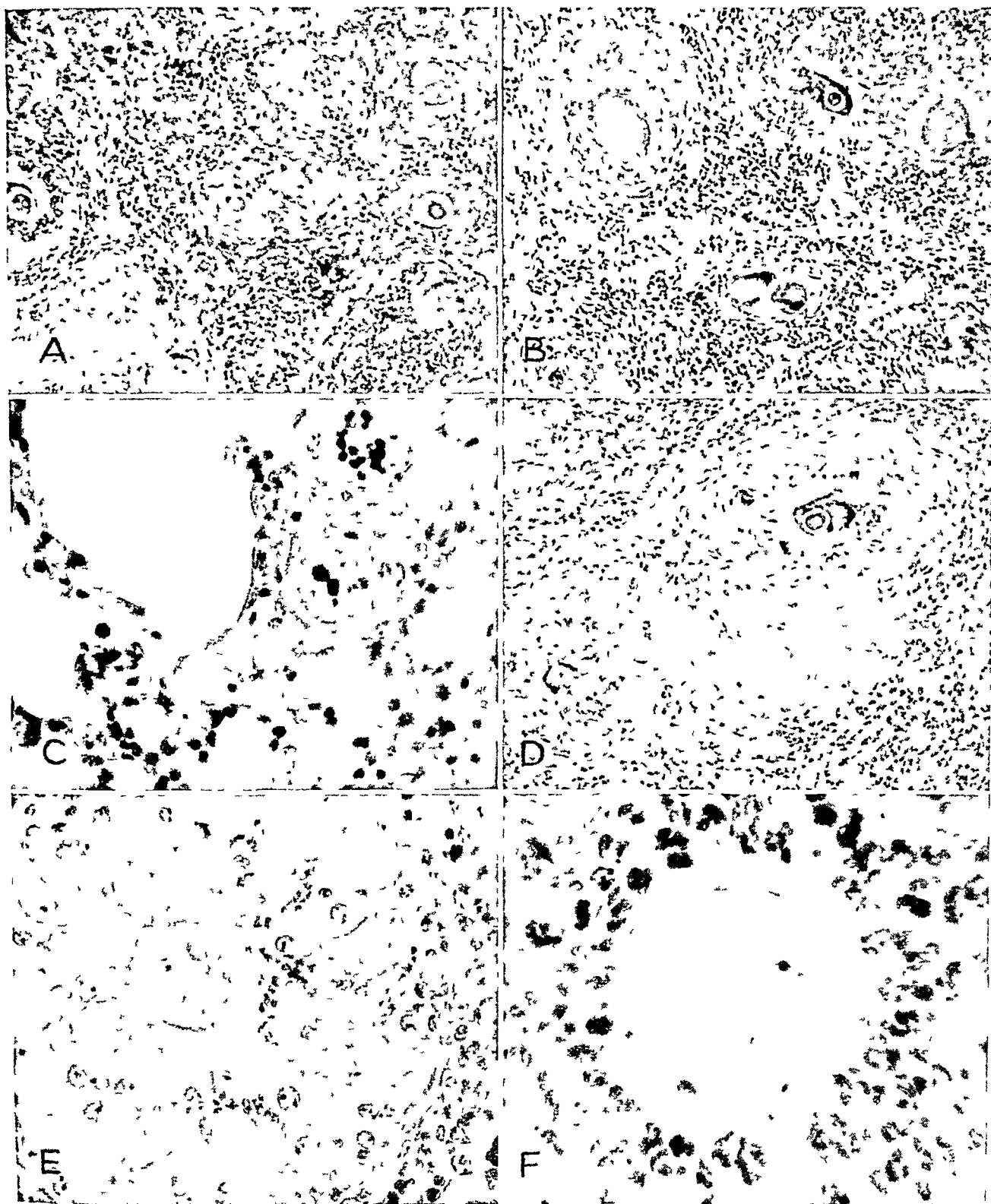
A few wild rodents (*Perognathus baileyi*, *Perognathus intermedius*, *Perognathus penicillatus*, *Dipodomys merriami*, *Dipodomys spectabilis*) were brought to the laboratory from the field stations and experimentally infected. Experimental studies involving these animals were inconclusive, and additional specimens have not been available for use. Hamsters and cotton rats were inoculated intranasally and guinea pigs intracardially, but these species appeared to be more resistant than white mice. In guinea pigs the fungus was observed by microscopic examination in the spleen, which was small, and in the lungs and kidneys, which grossly showed no abnormalities. The fungus was recovered in culture of material from guinea pigs killed one month after intracardial inoculation, but many of the fungous

cells showed degenerative changes and in animals saved for a longer period the fungus, although it could be found in sections of the lungs and the spleen, was no longer viable as determined by culture. Intratesticular inoculation of guinea pigs caused local inflammatory reactions and atrophy of the testicle but no progressive infection. Four goats were inoculated by dropping a suspension of spores into the glottis while they were under anesthesia induced with pentobarbital sodium and were reinoculated two weeks later by transpleural pulmonary inoculation. None of the animals showed pulmonary lesions. Attempts to produce lesions in 2 monkeys by intranasal instillations were not successful, but another monkey which received repeated inoculations of spore suspensions through the wall of the chest showed extensive changes, to be discussed in the section on pathology. Another monkey which received fungous spores by both routes also showed pulmonary lesions.

In the study of experimental *H. parvum* infection in white mice, approximately 200 of these animals have been examined histologically. However, the following description of the lesions produced is based largely on those observed in a group of 41 mice inoculated with strain 1093. Approximately half of this group received one inoculation, the other half received a second inoculation two weeks after the first. Since there was no recognized difference either in the extent or the character of the lesions or in the number of cells of the fungus between the group receiving only one inoculation and the group which received two inoculations, all 41 animals were used in studying the genesis of the pulmonary lesions. Groups of the animals were killed one, two, four, and five months after the original inoculation. For a description of earlier lesions (under one month), mice inoculated with fungous strain 1093 but from another experimental group were studied.

#### HISTOLOGIC OBSERVATIONS

The lungs of some mice examined from two to three weeks after intranasal inoculation showed a very few, quite small collections of mononuclear cells enclosing single spherical cells of the fungus, ranging in diameter from 5 to 10 microns. The wall of the fungous cell was refractile, thin and unstained, and the cytoplasm was basophilic and of uneven density, though not distinctly vacuolated. Other mice in this period showed pulmonary lesions as well developed and fungous cells as large as those seen in animals killed after one month. In the mice examined a month after inoculation, many cells of the fungus were seen, and most of them ranged from 25 to 30 microns in diameter. The walls of these fungous cells measured 3 to 4 microns in thickness and were oxyphilic and slightly anisotropic. The cytoplasm was less basophilic and less dense than that of the small fungous cells.



A, lung of a mouse four months after inoculation with spores of *H. parvum*, showing a group of granulomas and cellular infiltration of intervening tissue,  $\times 90$

B, group of granulomas in the lung of a monkey killed three months after transpleural pulmonary inoculation of *H. parvum*,  $\times 75$

C, lung of a mouse showing two cells of the fungus in different stages of growth,  $\times 300$  This mouse received two inoculations four weeks apart

D, larger lesion from the same lung shown in B,  $\times 85$

E, portion of a large mononuclear cell granuloma with centrally located cell of *H. parvum*,  $\times 430$

F, details of a cell of *H. parvum* in a granuloma,  $\times 575$  The nucleus and the nucleolus are distinct Note the clustering of polymorphonuclear leukocytes around the fungus

seen in some mice killed after two to three weeks. Numerous globules of fat were present in most fungous cells seen in animals killed one month or longer after inoculation. In tissue fixed in a solution of formaldehyde the cytoplasm of the fungus often appeared thready, granular or vacuolated. Some of the lungs were fixed in Bouin's fluid, dehydrated and infiltrated slowly with paraffin. In these, the cytoplasm of the fungus was more homogeneous. Most cells of the fungus, particularly if serial sections were made, showed a single oxyphilic nucleus, ranging from 5 to 7 microns in diameter, with a basophilic nucleolus, measuring 1.9 to 2.5 microns in diameter. A number of these large cells of the fungus were surrounded by a single layer of large mononuclear cells. Others were set in cellular nodules up to 100 microns in diameter, formed mainly of large mononuclear cells but showing also small numbers of lymphocytes. Rarely a few neutrophils separated the fungous cell from the enclosing mononuclear cells. Giant cells were rarely seen in such nodules. The fungous cells and cellular nodules were most numerous in the hilar portion of the lobe. In the few animals where they were fairly numerous, this area often showed the nodules touching one another or the alveoli between them filled with quite large mononuclear cells and a few irregularly scattered lymphocytes and neutrophils. A striking observation in these lungs was that of thick perivascular collars of lymphocytes. These lymphocytes were fairly densely packed and the collars of cells were sharply limited marginally. This infiltration was most pronounced around the larger vessels toward the hilus. It was also seen commonly in other areas, both adjacent to and distant from mononuclear cell nodules.

In the lungs of mice killed two months after inoculation, a few fungous cells were surrounded by only a single row of mononuclear cells, but generally the nodules were slightly larger than those seen after one month. Giant cells abutting on or enclosing the cells of the fungus were more frequent, and clusters of polymorphonuclears around the fungous cells were present in many nodules. In most instances a single cell of the fungus was seen at the center of the cellular nodule, however, larger, irregularly shaped monocytic masses were present which contained three or four irregularly placed fungous cells. In such a lesion lymphocytes and polymorphonuclears were sometimes fairly numerous. Lymphocytes usually were most numerous at the periphery of the lesions, rarely the nodules were formed almost entirely of these cells. The cells of the fungus were generally slightly larger,

occasionally measuring 40 microns. The walls of the fungous cells were also thicker and not infrequently had an outer basophilic lamina. This layer was sharply limited from the inner oxyphilic portion and generally was of even thickness, although occasionally a knobby excrescence was seen.

The pulmonary lesions of mice killed four months after inoculation were similar to those in mice killed after two months. However, the mononuclear cell masses enclosing the cells of the fungus showed less variation in size. Often they measured 200 microns and fungous cells surrounded only by a single row of mononuclear cells were rare. The cells comprising the nodules generally had much cytoplasm, which in many instances showed marked fine (foamy) vacuolation. Multinucleated giant cells, often completely enclosing the fungous cells, were seen with considerable regularity. In some animals they were present in practically all nodules, and occasionally a number of giant cells formed the bulk of an individual lesion. Polymorphonuclear leukocytes were present in greater numbers around the cells of the fungus and were almost constantly present. Occasionally a nodule was formed of about equal numbers of polymorphonuclears and mononuclears, completely intermingled. Perivascular lymphocyte infiltration was still prominent four months after inoculation and the apparent fusion of nodules, due to the presence of large mononuclears in inter-nodular alveoli, was more striking. In some mice the medial half of one or more pulmonary lobes was largely "consolidated" in this manner. Occasionally (2 of 9 mice) there was slight to moderate deposition of collagen fibers in the outer part of the nodules. Here, concentrically arranged spindle-shaped fibroblasts were seen, giving more definite circumscription to the nodules than is usually observed. The cells of the fungus showed no further increase in size, but their walls were thicker. The basophilic outer lamina generally was of greater thickness than observed earlier and more uneven, and in the process of sectioning the tissue this outer layer was sometimes fractured. In a few animals the walls of most fungous cells were entirely basophilic, but the cytoplasm generally was not recognizably altered. Rarely the cytoplasm was oxyphilic and apparently necrotic. The thick walls of a number of the fungous cells seen at various intervals, but most often between two and four months, appeared to be formed of from two to four concentric layers, each exhibiting a distinctly different degree of oxyphilia.

The degree of involvement and the appearance of the lesions in the lungs of mice killed five months after inoculation showed little change from what was seen at four months. Although an occasional necrotic fungous cell was seen, there was no apparent significant reduction in their number nor any lessening of reaction to them.

Minor alterations were occasionally observed in other organs. These appeared unrelated to the experimental infection.

In an experiment using strain 234 of *H. parvum* as the source of the inoculum, 18 mice received only one inoculation. A single fungous cell was seen in the lungs of 1 of these animals. Of 20 mice receiving a second inoculation two weeks after the first, 4 showed involvement similar to that following inoculation with strain 1093. In 1 mouse the lesions were extensive.

The 2 monkeys given spores of the fungus by intranasal instillation showed no pulmonary lesions. However, a monkey receiving the spores by transpleural pulmonary inoculation showed extensive involvement of the inoculated right lung and similar but less extensive involvement of the left. This monkey was killed ninety days after inoculation. The alveolar structure of the lung was almost completely obliterated by thickening of septums resulting from cellular infiltration, mainly lymphocytes and plasma cells. In such areas granulomas up to 400 microns (average about 200 microns) were numerous. The granulomas were formed mainly of polygonal epithelioid cells and usually showed one, occasionally more, large giant cells (one measured 300 microns). The visceral, parietal and interlobar pleura on the right side was thickened, fibrous and adherent and showed numerous granulomas. There were present in the peribronchial and mediastinal lymph nodes scattered granulomas similar to those in the lung.

Another monkey was given a suspension of spores by nose and after a lapse of one hundred and eighteen days a similar suspension by transpleural pulmonary inoculation, it was killed thirty-five days later. The pulmonary infection was essentially similar to that just described. However, the granulomas appeared older. Many of the epithelioid cells were spindle shaped, the giant cells were smaller and more numerous, and there was moderate fibrosis in a number of the lesions. Moreover, most of the cells of the fungus were dead, empty fungous capsules being fairly numerous. This would suggest that most of the granulomas resulted from the intranasal inoculation rather than the transpleural pulmonary inoculation done thirty-five days before death.

#### COMMENT

The description of the lesions produced by *H. parvum* in the lungs of mice and monkeys has been given in some detail to show that the reaction is progressive for a period of four to five months, and for the purpose of comparing them with the spontaneous lesions occurring in wild rodents. It is true that there is no evidence to show that propagation of the fungus took place in the experimental animals, and in that sense a progressive infection was not produced. It is evident that the fungus undergoes considerable growth, remains viable for a lengthy period and produces a considerable reaction. Although serial sections were not made, the examination of thousands of experimentally produced granulomas indicates that each individual lesion usually results from a single fungous cell located near its center. That the lesion is more than a foreign body reaction is indicated by a number of features. First, a pneumonic reaction occurs in some animals shortly after the introduction of the spores (killed spores introduced in a similar manner failed to produce lesions). Second, a marked perivascular lymphocyte infiltration occurs not only in the area of the granulomas but at some distance from them, not infrequently in areas which show no other alteration. Third, the polymorphonuclear leukocyte infiltration is prominent in some of the granulomas, particularly immediately adjacent to the cells of the fungus, and in the older lesions. Lastly, the lesions exhibit a slow but steady growth for four or five months.

In the description of the fungous cells, the thickness of the wall was given and various oxyphilic and basophilic layers were mentioned. This separation into different layers on the basis of staining properties is likely artificial and probably represents only a variation in the  $pH$  level of different portions of the wall. However, it is possible that part of the apparent wall is contributed by the surrounding animal tissue. This wall swells to an unusual degree when lung tissue containing the fungus is macerated and mounted in 10 per cent sodium hydroxide.

It is of interest to compare briefly the lesions seen in the experimental animals with those occurring in naturally infected wild rodents. In earlier publications<sup>1</sup> two types of lesions were described. One type was a small, irregularly shaped collection of large mononuclears usually enclosing only one cell of the fungus. A second type was characterized by well organized granulomas up to 2 mm in diameter, usually containing numerous cells of the fungus. The latter type of lesion was not reproduced in the experi-

mental animal. We suggest that this was due to the failure of the fungus to propagate in the lungs of the experimental animal. This belief is supported by certain observations in the study of the naturally infected wild mice (*Perognathus*). Evidence presented previously<sup>1a</sup> indicates that the fungous cells in some of these granulomas were the progeny of *H. parvum*. In a few of the naturally infected wild mice showing well formed granulomas with abundant fungous cells, there occurred small (up to 200 microns) satellite nodules in contact with the wall of the larger granuloma. These small nodules resembled in many respects the granulomatous lesions seen experimentally. Likewise they were formed around single, centrally located fungous cells.

Endosporulation in *Haplosporangium* has not been observed in experimentally infected mice, and there appears to be a discrepancy in this respect between the field and the experimental data which we have not yet been able to explain. Endosporulating fungous cells which closely resembled *Coccidioides* were found in the sectioned lungs of naturally infected *Perognathus*. From some of these animals *Haplosporangium* only was isolated from the portion of lung cultured. Further *Haplosporangium* only was isolated from 18 *Perognathus* mice presenting granulomas, most of which contained numerous large fungous cells which were not sporulating but presumably were the result of earlier endosporulation. It is possible that in these, as in some cases observed, an animal was infected by both fungi and that the portion of lung cultured contained only lesions caused by *Haplosporangium*, while conversely the portion sectioned contained only lesions caused by *Coccidioides*. It seems more probable, however, that in the lungs of *Perognathus* *Haplosporangium* actually reproduces by endosporulation in a manner similar to that of *Coccidioides*. These rodents, as previously pointed out, appear to bear an unusual host-parasite relationship to *Coccidioides*, and it is reasonable to suppose that their adjustment to the infection resulting from inhalation of conidia of *Haplosporangium* also differs from that of the laboratory animals we have examined. It is probable, in view of the type of lesions already produced in experimental animals, that if a more suitable animal can be found or if the suscepti-

bility of experimentally infected white mice can be increased, the life cycle of *Haplosporangium* in experimentally infected animals can be completed.

#### SUMMARY

Of all the animals experimentally infected with *H. parvum* (mice, guinea pigs, rats, monkeys, hamsters and goats), mice appeared to be the most satisfactory and were used in the study of lesions produced by this fungus. Intranasal instillation of a suspension of spores was the inoculation method of choice. In mice killed two weeks after inoculation the pulmonary lesions were small and formed of compactly disposed large mononuclear cells. Such a lesion enclosed a single round cell of the fungus, measuring 5 to 10 microns in diameter. In animals killed one month after inoculation the lesions measured up to 100 microns and were formed largely of mononuclear cells with a few lymphocytes and polymorphonuclear leukocytes. The fungous cells measured 25 to 30 microns and had fairly homogeneous basophilic cytoplasm. Each fungous cell exhibited a single nucleus and a distinct nucleolus. Mice were studied at intervals up to five months after inoculation. From these it was evident that the lesions grew slowly for about four months. The individual lesion at this stage was generally round, fairly distinct marginally and measured up to 200 microns. Giant cells, which appeared earlier, were fairly numerous at four months. Fungous cells up to 35 microns were frequently seen and had thick walls, often appearing laminated. Evidence of propagation of the fungus in the tissue was not observed.

The lesions occurred most often in the medial half of the pulmonary lobes, and this portion was often consolidated by contiguous or closely grouped granulomas and had an exudate of large mononuclear cells in the intervening alveoli. Organs other than lungs showed no lesions which appeared related to the experimental infection.

An experiment planned to permit study of a possible immunologic relationship between *H. parvum* and *C. immitis* showed that mice inoculated with *H. parvum* were as susceptible to *C. immitis*, when inoculated three weeks later, as were control mice.

# PANCREATIC NECROSIS IN ELECTRIC SHOCK

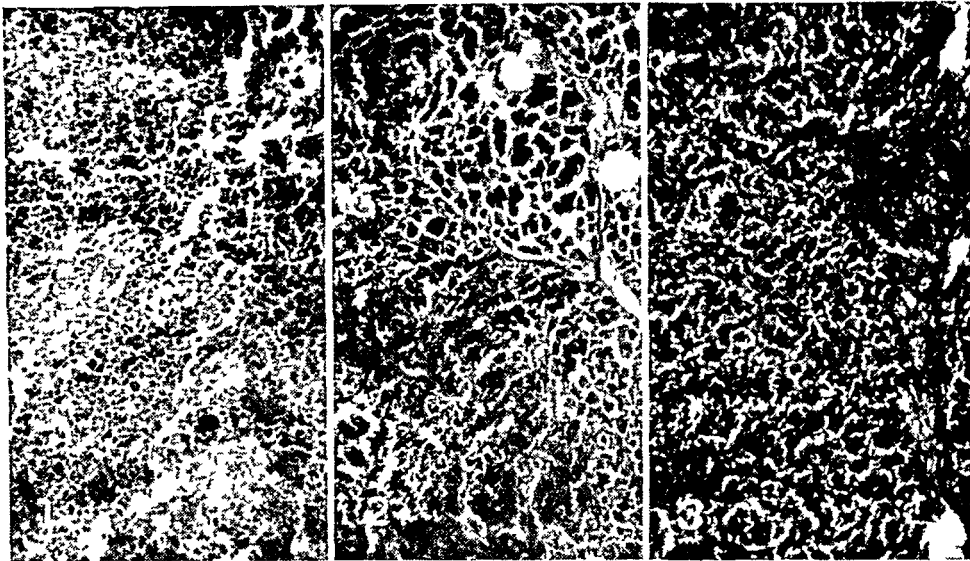
MAJOR ALFRED M GLAZER

MEDICAL CORPS, ARMY OF THE UNITED STATES

During the past year 3 cases of extensive pancreatic necrosis associated with fatal electric shock have been studied. Most articles on this subject do not mention pancreatic changes. Jaffé<sup>1</sup> in an excellent review of electropathology made no mention of them, nor is there any reference to pancreatic changes in electric shock in MacCallum's<sup>2</sup> "Textbook of Pathology." An extensive article by Sirolli,<sup>3</sup> however, does describe similar changes in animals dying of electric shock.

## REPORT OF CASES

CASE 1—A soldier was telephoning during an electric storm June 10, 1943. He suddenly called out, "I have been struck," then walked out of the tent and collapsed. He was given stimulants and artificial respiration but died almost immediately. An autopsy was made four hours after death. The pancreas was of normal size and shape. Its head and neck appeared normal. Its body and tail, however, were extremely flabby and on section appeared slightly duller than normal and markedly congested. Microscopic sections of the pancreas showed marked variations. Some areas presented



Photomicrographs of pancreas showing various degrees of necrosis without cellular reaction. Hematoxylin and eosin, paraffin sections,  $\times 100$

In view of the frequency of electric shock of a major or minor degree, wider recognition of possible pancreatic necrosis is indicated. In non-fatal cases minor pancreatic changes may occur, which could be determined by studies of serum amylase. Awareness of this possibility is important for early diagnosis and treatment and for the recognition of possible late sequelae. Minor pancreatic changes may give no immediate symptoms but may later cause pancreatic insufficiency. This is especially important in industrial cases from a medicolegal standpoint.

normal tissue, some slight necrosis with loss of nuclear structure and disintegration of cellular boundaries, and some complete necrosis with total loss of normal structure and without cellular reaction (fig 1). There were also areas of apparent hemorrhage. The other abnormalities of note were extensive burns of the skin, focal areas of hemorrhage in the myocardium and the epicardium, focal areas of hemorrhage in the lungs and extreme congestion of the kidneys, the spleen, the brain and the adrenal medulla.

CASE 2—A soldier was standing guard July 19, 1943 during an electric storm. A terrific burst of lightning occurred, and he was seen to lean against a barbed wire fence. A moan was heard, and on examination two or three minutes later he was pulseless and not breathing. All types of stimulants and prolonged artificial respiration were given to no avail. An autopsy was performed sixteen hours later, the body having been placed in a refrigerator immediately after death. The pancreas

1 Jaffé, R. H. Arch Path 5 837, 1928

2 MacCallum, W. G. A Textbook of Pathology, ed 7, Philadelphia, W. B. Saunders Company 1940

3 Sirolli, M. Arch ital di chir 33 333, 1933

was of normal size and shape. Its head, neck and body were normal. Its tail, however, was much flabbier and duller than normal and on section was markedly congested. Microscopic sections of tissue from the head, the neck and the body were normal. Sections from the tail presented extensive hemorrhage with extensive apparent coagulation necrosis without any cellular reaction (fig 2). The other changes of note were extensive acute congestion of all sections of the central nervous system with slight to moderate perivascular hemorrhage.

CASE 3—A soldier was found dead in a bathtub on May 27, 1944. It was surmised that while taking a bath he attempted to plug in a radio at a wall outlet and was electrocuted. An autopsy was performed eight hours later, the body having been placed in a refriger-

ator. The pancreas was of normal size and shape. The entire pancreas was extremely flabby and duller than normal. The cut surface was congested and had a speckled appearance, which was most marked in the tail. Microscopically, the pancreas presented areas of slight, moderate and complete necrosis without cellular reaction. There were also areas of slight hemorrhage (fig 3). The other alterations of note were acute congestion of the central nervous system with focal areas of perivascular hemorrhage.

#### SUMMARY

Cases of electric shock in which pancreatic necrosis is present are of possible clinical and medicolegal significance.



# CORRELATION BETWEEN CHEMICAL AND MORPHOLOGIC ALTERATIONS IN EXPERIMENTAL ATHEROSCLEROSIS

O J POLLAK, MD

TAUNTON, MASS

The morbid anatomy of the various stages of experimental atherosclerosis is well known. For this knowledge physicians and research workers are greatly indebted to Leary<sup>1</sup>. It has been sufficiently proved that cholesterol is the chief causative agent in human as well as in experimental atherosclerosis.

The results of a study of the correlation between the amount of cholesterol ingested, the level of the blood cholesterol and the presence and the degree of atherosclerotic lesions in rabbits are presented.

## MATERIAL AND METHODS

Sixty rabbits, weighing between 2 and 4 Kg, were used for the experiment, 40 of these rabbits were fed cholesterol, and 20 served as controls. There were equal numbers of males and females in each group.

The animals were kept on a diet of fresh vegetables. Each of the cholesterol-fed animals was given daily 0.5 Gm of powdered cholesterol in a gelatin capsule.<sup>2</sup> Venous blood was drawn weekly in the morning hours for the estimation of cholesterol, and Bloor's<sup>3</sup> method adapted for a photoelectric colorimeter was used.

## OBSERVATIONS

The initial level of the blood cholesterol in 60 rabbits was between 108 and 170 mg per hundred cubic centimeters, the average was 135 mg. There was no appreciable difference in either sex. The maximum individual variation of blood cholesterol level in the 20 control animals throughout an observation period of three months was 55 mg per hundred cubic centimeters.

The cholesterol-fed animals were divided into three groups, 10 received 30 Gm of cholesterol in sixty days, 10 others 40 Gm in eighty days and 20 received 50 Gm in one hundred days. The animals were killed after the termination of their feeding period, the controls, at the end of the experiment.

A study of the weekly blood cholesterol values of each rabbit revealed considerable variation from one animal to another. Those with similar blood cholesterol

curves were combined and placed into four distinct groups (A, B, C and D).

Group A (fig 1) consisted of 7 rabbits (17.5 per cent of all 40 cholesterol-fed animals), 3 males (15 per cent of 20 males) and 4 females (20 per cent of 20 females). The blood cholesterol curves for the sexes were alike. There was a slow gradual rise which after five weeks reached a low maximum of about 400 mg per hundred cubic centimeters. From this maximum point the curve fell, and at the end of twelve weeks the blood cholesterol value was normal.

Group B (fig 2) consisted of 14 rabbits (35 per cent), 9 males (45 per cent) and 5 females (25 per cent). The curves for the sexes were similar. They rose slowly and steadily and at the end of fifteen weeks reached a low maximum of 430 mg per hundred cubic centimeters of blood.

Group C (fig 3) consisted of 9 rabbits (22.5 per cent), 4 males (20 per cent) and 5 females (25 per cent). The curves for the sexes were much alike. There was a comparatively quick rise in the first seven weeks, the blood cholesterol reaching about 600 mg per hundred cubic centimeters. This was followed by a temporary slight decline up to the twelfth week. In the third phase the curves ascended again to the formerly reached maximum.

Group D (fig 4) comprised 10 animals (25 per cent), 4 males (20 per cent) and 6 females (30 per cent). For each sex there was a steep curve which reached a first high value of about 700 mg per hundred cubic centimeters within six weeks. The curve for the females continued upward with occasional falls. That for the males showed a temporary rapid decline which reached its lowest point in the ninth week at 500 mg per hundred cubic centimeters. There was, however, a third phase in which the curve for the males again ascended to a high level. The maximum value which was reached at the end of the experiment was over 800 mg in the curve for the males and almost 1,000 mg in that for the females.

The curves for the first two groups (A and B) and those for the second two groups (C and D) had some common characteristics. The first two curves rose slowly and reached a low maximum, the second two curves rose faster and reached higher values. Group A showed an irreversible and group C a temporary drop of the values whereas the other two curves showed a more or less steady increase. The type of initial rise was evident from a comparison of the length of time between the onset of the experiment and the point at which the initial blood cholesterol value was doubled. It took four weeks for A, three weeks for B and C and only two weeks for D. On the whole, the curves for the sexes were similar. The number of female rabbits in each of the four groups was almost the same. There were only 15 per cent of male animals in group A, in group B there were 45 per cent, and equal numbers of male animals were present in groups C and D.

Figure 5 represents a summary of the average weekly blood cholesterol estimations of all 40 cholesterol-fed

From the Research Division of the Taunton State Hospital

This work was aided by a grant from the Department of Mental Health of the Commonwealth of Massachusetts for research in arteriosclerosis. This paper is the first of a series.

1 Leary, T Arch Path 17 453, 1934, 32 507, 1941, 37 16, 1944

2 Pollak, O J Arch Path 37 337, 1944

3 Bloor, W R J Biol Chem 24 227, 1916



rabbits The curves for the sexes, as well as the average combined curve for all 40 animals, increased gradually with occasional falls Only in the last weeks of the experiment were the curves dissimilar The blood cholesterol values of the male rabbits ascended to a higher level than those of the females

The graphs included the cholesterol values for all the animals regardless of the duration of their feeding period A certain number of rabbits were eliminated after the intake of 30 or 40 Gm of cholesterol Therefore, the first part of each curve presented a summary of values for a larger number of animals than did the last part

degree and the extension of the atherosclerotic process increased with the intake of cholesterol

The gross and the microscopic atherosclerotic lesions observed in the rabbits were arbitrarily rated according to their degree and extension An accumulation of foam cells in the subendothelial layer in a single plaque was considered the first sign of atherosclerosis (1 degree) Thickening of the intima proper, fraying of the inner elastica and involvement of a larger area were indications of a more advanced process (2 degrees) Further spread of the lesion, the appearance of young fibrous tissue progressing from the subintimal layer toward the intima, and hyperplasia of fibrous and elastic

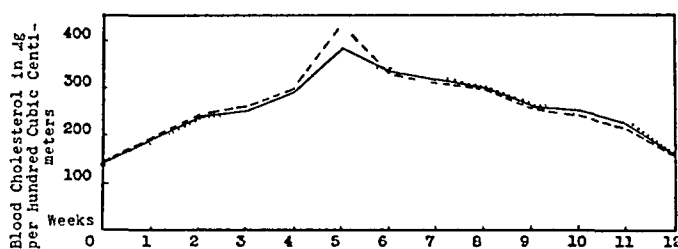


Fig 1—Blood cholesterol curves of group A (males (—○—), females (---○---), combined average (——))

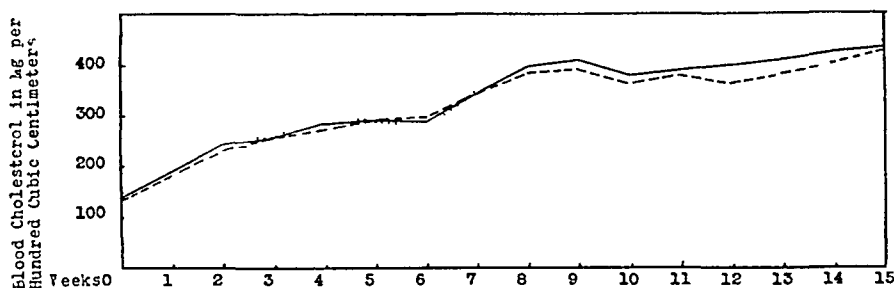


Fig 2—Blood cholesterol curves of group B (males (—○—), females (---○---), combined average (——))

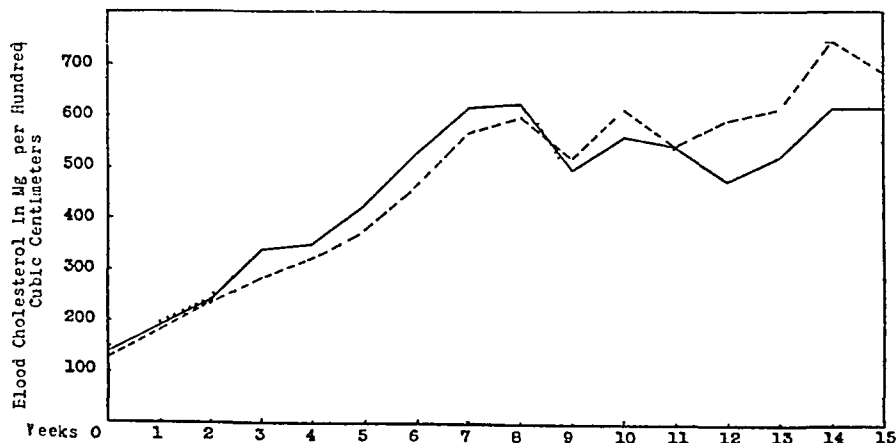


Fig 3—Blood cholesterol curves of group C (males (—○—), females (---○---), combined average (——))

The type of curve did not depend on the initial blood cholesterol value, nor did it depend entirely on the amount of cholesterol ingested After an intake of 30 Gm of cholesterol, the lowest, average and maximum blood cholesterol values were 308, 586 and 900 mg per hundred cubic centimeters, respectively After ingestion of 50 Gm 443 mg was the lowest, 761 mg the average and 1,216 mg the highest Some rabbits had a higher blood cholesterol value after a lower intake, others, a relatively lower value after ingestion of more cholesterol

A relatively large number of rabbits had atherosclerotic lesions after the intake of 30 Gm of cholesterol, while some rabbits did not respond after 50 Gm The

elements of the media causing thickening of the wall represented the next degree of change (3 degrees) The highest degree of atherosclerosis encountered in the experiments was characterized by involvement of a wider area, hyalinization of the newly formed fibrous tissue, mucoid degeneration of the foam cells, relative reduction in the depth of the media and marked hyperplasia and fibrosis of the external elastica and adventitia (4 degrees) These four degrees of pathologic changes were evident when the photomicrographs of the lesions (fig 6 B, C, D and E) were compared with one of the aorta of a control animal (fig 6 A)

Of 40 cholesterol-fed rabbits, 22 (55 per cent) presented various degrees of atherosclerosis Among these

there were 12 male and 10 female animals. In one half of the rabbits sclerotic lesions developed in only one location, mostly in the aorta, frequently in the coronary vessels, less often in the renal or the carotid arteries. The other half of the animals had atheromatosis of several of the branches of the vascular tree. In this group the aorta and the carotid arteries were almost always involved. The degree of atherosclerosis increased with the extent and the multiplicity of the lesions. The aorta and the carotid arteries were affected

cholesterol per hundred cubic centimeters of blood. There were 5 animals (12 per cent) with blood cholesterol values of 788, 795, 846, 870 and 900 mg per hundred cubic centimeters, which did not show the slightest sign of atherosclerosis on microscopic examination of all organs. These rabbits belonged to group C, C, D, C and D, respectively.

The accompanying table shows the presence and the degree of atherosclerotic changes in the four groups of rabbits (A, B, C and D).

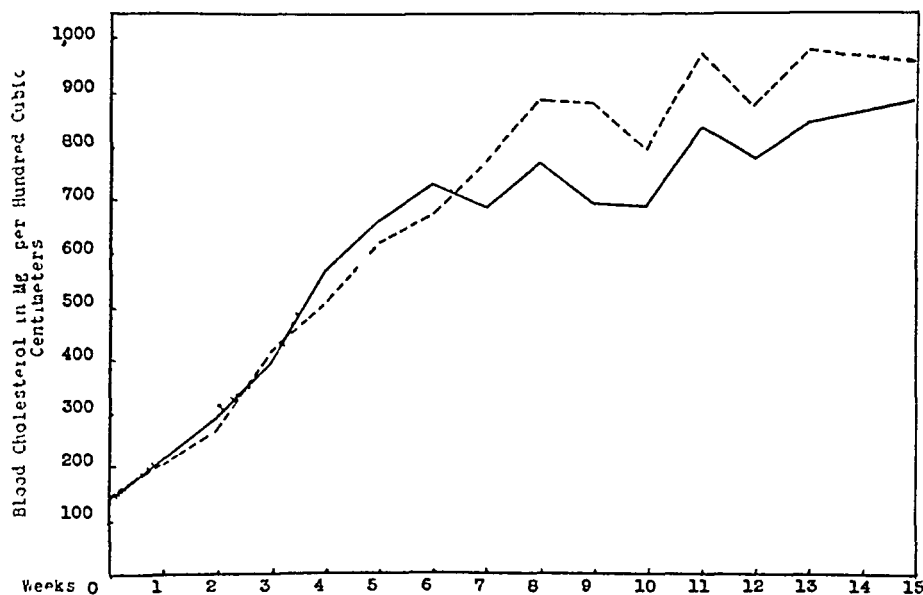


Fig 4—Blood cholesterol curves of group D (males (—), females (---), combined average (- - -))

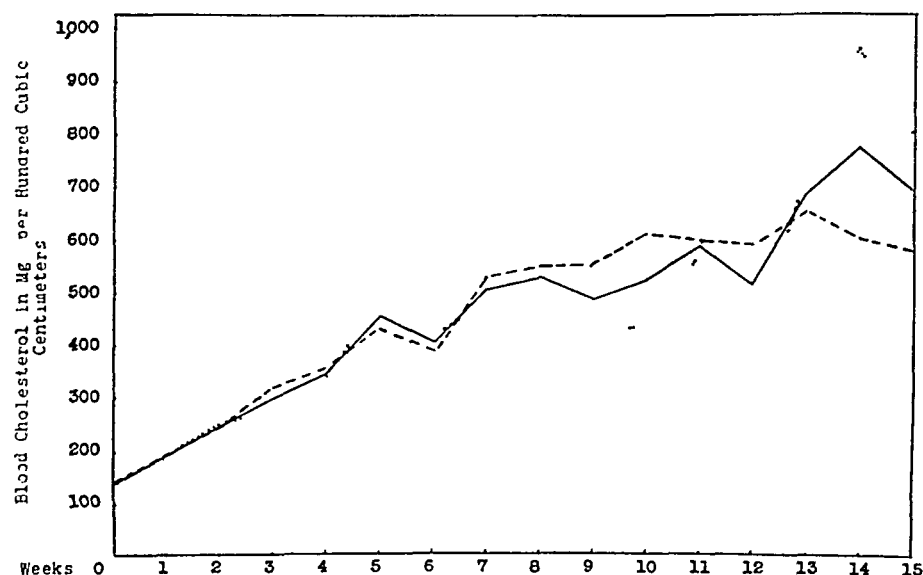


Fig 5—Summary of blood cholesterol values for groups A, B, C and D (males (—), females (---), combined average (—))

twice as frequently as the coronary or the renal vessels. In two thirds of all animals with sclerotic lesions the aorta showed microscopic changes. However, only 4 rabbits presented gross evidence of atherosclerosis in the form of yellow streaks and elevated patches in the descending aorta.

The first evidence of atherosclerosis was found in a rabbit of group A whose maximum blood cholesterol value was 300 mg per hundred cubic centimeters. The highest degree of atherosclerosis visible at gross inspection was found in rabbits with blood cholesterol levels of 615, 846, 1,200 and 1,215 mg per hundred cubic centimeters. These animals belonged to groups B, C, D and D, respectively. The single rabbit with absolute maximal lesions was the one with 615 mg of

#### Response of Rabbits to Cholesterol Feeding

Group	Total No of Rabbits	Rabbits with Response		Rabbits Showing Atherosclerosis of Given Degree			
		Negative	Positive	1°	2°	3°	4°
A	7	5	2	2	0	0	0
B	14	5	9	2	3	3	1
C	9	4	5	1	3	0	1
D	10	4	6	1	1	2	2
Totals	40	18	22	6	7	5	4

The number of rabbits in which atherosclerosis developed was small only in group A. The degree of the

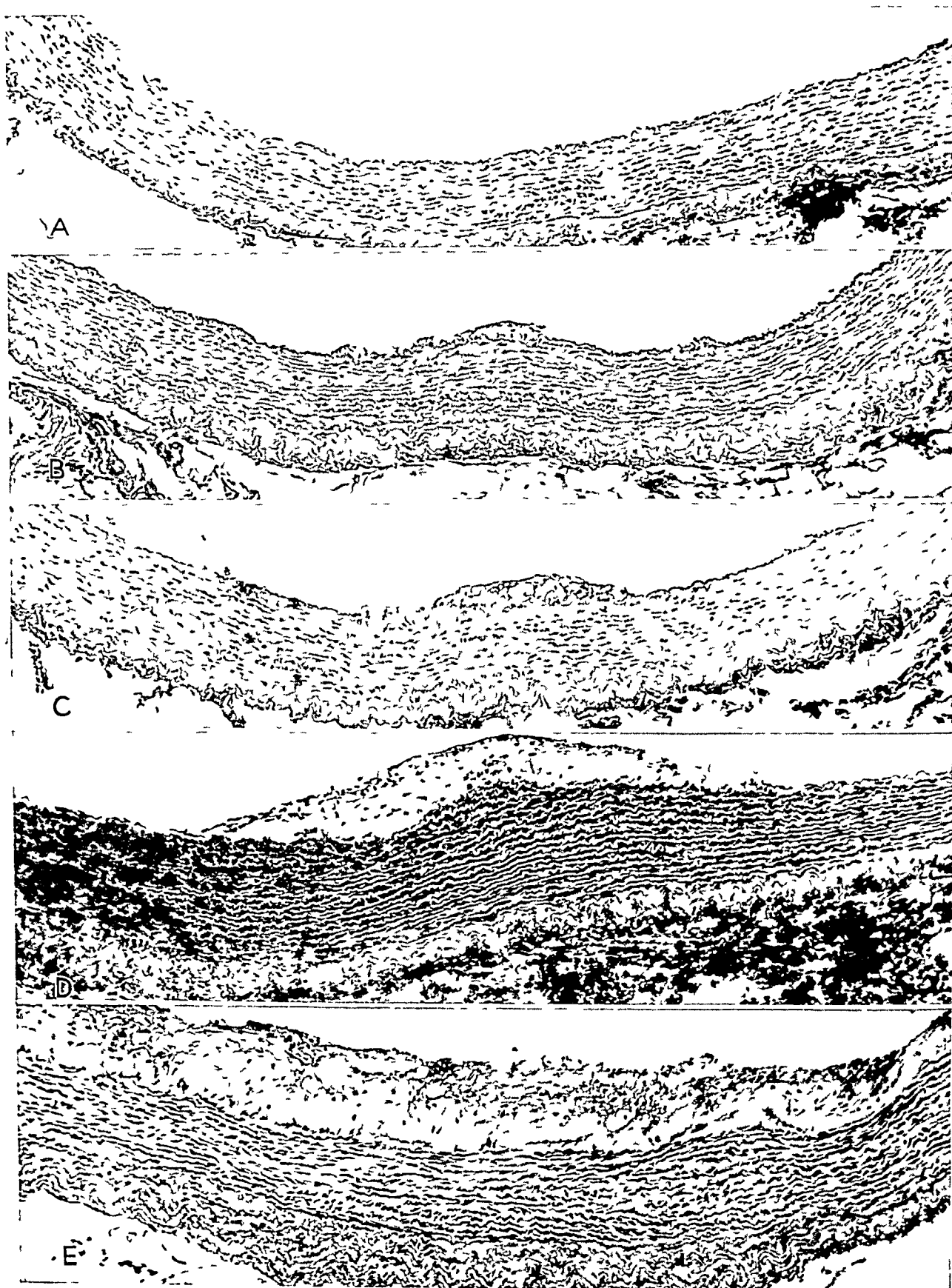


Fig 6—*A*, normal aorta (control) *B*, *C*, *D* and *E*, degrees 1, 2, 3 and 4 of experimental atherosclerosis of the aorta. Fixation in solution of formaldehyde, rapid trichrome stain,  $\times 120$

lesions was moderate. This group of animals had a temporary and slight increase of the blood cholesterol. The rabbits of the other groups (B, C and D) showed a more uniform reaction as to presence and degree of atherosclerosis, although the slope of the three curves was different, that is, slowly ascending with a low maximum in B, steeper and much higher in C and D.

#### COMMENT

There are individual responses of rabbits to cholesterol feeding. This is obvious from the distinct types of curves of the blood cholesterol values and from the maximum elevations of these curves.

While controls maintain a constant blood cholesterol level, rabbits fed cholesterol show hypercholesteremia. The degree of this condition depends on the individual response of the rabbit rather than on the amount of cholesterol ingested, since animals fed the same amount of cholesterol react differently.

The fact that only a small percentage of animals in group A of the series presented atherosclerotic lesions can be interpreted from the type of curve. An explanation of the absence of atherosclerosis in some animals of the other groups (B, C and D) and especially in some rabbits whose blood cholesterol reached high levels is rather difficult. One has to assume that there are considerable differences in the ability of animals to metabolize cholesterol and

that this quality is responsible for the variability of the length of time of the latent period. There is a probability that a number of the cholesterol-fed rabbits which did not respond with atherosclerosis would have shown lesions on continuation of the experiment.

The degree of atherosclerosis, although surprisingly high in some animals with relatively low blood cholesterol values, is on the whole parallel with the degree of hypercholesteremia and indirectly related to the amount of ingested cholesterol.

#### CONCLUSIONS

Feeding of cholesterol to rabbits causes temporary or permanent hypercholesteremia of a varying degree. The type of reaction depends on the individual ability to metabolize cholesterol. The more cholesterol ingested the greater is the probability of hypercholesteremia.

Hypercholesteremia is not necessarily followed by atherosclerosis, as the threshold for cholesterol of the rabbits differs from one animal to another. The higher the blood cholesterol value the more likely it is that atherosclerosis will develop.

Once the defense mechanism is broken and atherosclerosis develops, the degree and the extensiveness of the process will increase with the rise of the blood cholesterol level.

# ATTEMPTS TO PRODUCE CEREBRAL ATHEROSCLEROSIS

O J POLLAK, M D

TAUNTON, MASS

Experimental production of cholesterol atherosclerosis in rabbits has been frequently reported. The vast majority of investigators enumerate many different sites of sclerotic lesions but fail to mention the cerebral vessels<sup>1</sup>. The few authors who describe the cerebral arteries point out the absence of atherosclerosis<sup>2</sup>.

The goal of the present study was to produce atherosclerosis in the cerebral vessels of cholesterol-fed rabbits by impairment of the cerebral circulation.

The setup of the experiments is shown in the accompanying table.

Group A were controls for the entire series of rabbits. No operations were performed on, and no injections were given to, these animals. As in the other groups, 4 rabbits were fed cholesterol and 2 were not. The animals of group B had both internal carotid arteries permanently constricted by ligation. Group C consisted of rabbits which had both jugular veins ligated. The rabbits of group D had the left internal carotid artery and the right jugular vein tied.

The carotid arteries were tied just above their origin and the jugular veins at the level of the thyroid gland. All ligations were performed one week before the chole-

## Outline of Experiments

Group	Ligations *	Substance Injected	Rabbits Receiving Cholesterol in Given Amount				Total Number of Rabbits
			0 Gm	30 Gm	40 Gm	50 Gm	
A			2	1	1	2	6
B	A A		2	1	1	2	6
C	V V		2	1	1	2	6
D	A V		2	1	1	2	6
E		Epinephrine hydrochloride	2	1	1	2	6
F	A A	Epinephrine hydrochloride	2	1	1	2	6
G	A V	Epinephrine hydrochloride	2	1	1	2	6
H		Histamine phosphate	2	1	1	2	6
I	A A	Histamine phosphate	2	1	1	2	6
J	A V	Histamine phosphate	2	1	1	2	6
All			20	10	10	20	60

\* A A indicates that both carotid arteries were tied. V V that both jugular veins were tied. A V that the left carotid artery and the right jugular vein were tied.

## MATERIAL AND METHODS

Each of 40 rabbits was fed daily 0.5 Gm of cholesterol in a gelatin capsule,<sup>3</sup> and 20 rabbits served as controls. The animals were kept on a diet of fresh vegetables. Of the cholesterol-fed rabbits, 10 received 30 Gm in sixty days, 10 others 40 Gm in eighty days and 20 50 Gm in one hundred days. The animals were killed after the termination of their feeding period, the controls, at the end of the experiment. There were equal numbers of males and females in both groups.

From the Research Division of the Taunton State Hospital.

This work was aided by a grant from the Department of Mental Health of the Commonwealth of Massachusetts, for research in arteriosclerosis. The paper is the second of the series.

1 Schoenheimer, R. Virchows Arch f path Anat 249 1, 1924. Leary, T. Arch Path 17 453, 1934.

2 Cowdry, E. V. Arteriosclerosis. A Survey of the Problem, New York. The Macmillan Company, 1933, pp 305-306. Duff, G. L. Arch Path 22 161, 1936.

3 Pollak, O. J. Arch Path 37 337, 1944.

terol feeding started. There was a high mortality following operation on both arteries. The animals died from convulsive shock immediately after the arteries were ligated. No death resulted from any other type of operation.

Each animal in groups E, F and G received a total of 2.56 mg of epinephrine hydrochloride in a 1,000 solution. Intravascular injections were given above the ligatures weekly during the last seven weeks of the experimental period. The individual doses increased gradually from 3 to 9 minims (0.18 to 0.55 cc) per injection. The animals in group E were given only epinephrine hydrochloride. Group F rabbits received injections and had both carotid arteries tied, and group G rabbits had one artery and one vein ligated.

The animals in groups H, I and J were treated in every respect the same way as the rabbits in the previous 3 groups (E, F, G) with the exception that they were given injections of histamine phosphate instead of epinephrine hydrochloride.

## CLINICAL OBSERVATIONS

The body weights and temperatures of the rabbits were recorded weekly, their behavior was constantly



Fig 1—*A* and *B*, atherosclerosis of the carotid artery ( $\times 60$ ) *A*, below ligature, *B*, above ligature *C*, and *D*, response of large cerebral vessels to ligation (basilar artery,  $\times 220$ ) *A*, control animal (no ligation), *B*, widening of the vessel, with stretching and thickening of the internal elastica, after ligation of the left carotid artery and the right jugular vein Fixation in solution of formaldehyde, rapid trichrome stain

watched, and shortly before their death the eyegrounds were examined

Half of all animals gradually lost weight, mainly those with operations on blood vessels. The weights of the other half remained constant or increased. In rabbits which were not fed cholesterol a gain of weight was more common. The rectal temperatures ranged between 101.1 and 103 F. The rabbits readily ingested their food and the cholesterol. Some animals were resistive at first, but once they learned to devour the capsules with cholesterol, no further difficulties were encountered. The rabbits displayed no obvious abnormal behavior in their cages. The rabbits with ligations of arteries were first excited and later dull, probably because of a temporary circulatory disturbance. The animals became alert when some one entered the room, and several snapped at the attendant while he fed them. The cholesterol-fed rabbits lost interest in sex. Animals of opposite sex placed in the same cage did not approach each other. The examination of the eyeground did not yield any positive observations on retinal vessels.

Sections of the brains were prepared in such manner as to allow observations of all parts of the brain and all the branches of the vascular tree. Sections were stained with hematoxylin and eosin, Weigert's stain for elastic tissue, Pollak's trichrome stain and Nissl's stain for nerve cells.

The observations largely support the views of Leary<sup>5</sup> against other theories<sup>6</sup> of atherosclerosis. Of the cholesterol-fed rabbits, 52 per cent had atherosclerotic lesions in various locations. Typical patches were visible in the thoracic aorta in 4 rabbits. Microscopically, involvement of the aorta was observed in 14 animals. Sclerotic lesions were seen in the carotid arteries of 10 rabbits, in the coronary arteries of 7 and in the renal arteries of 6. Occasionally the arteries of the spleen, the lung, the pancreas and the testicle showed initial sclerosis. The degree of atherosclerosis advanced with the multiplicity of lesions.

With the exception of the ligation of carotid arteries, the operations and the injections did not influence the

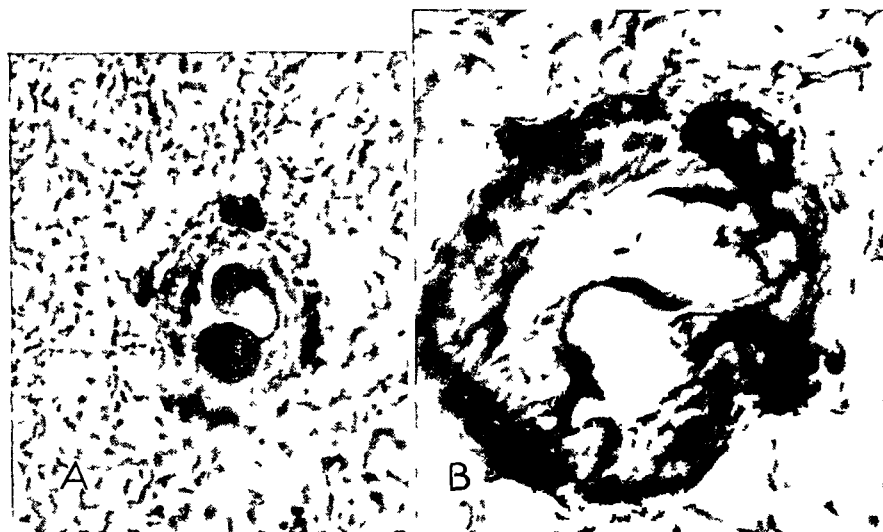


Fig 2—Response of small cerebral vessels to ligature (intracerebellar artery,  $\times 1,000$ ). A, control animal (no ligation), B, dilatation and hypertrophy of the vessel, edema of the wall and perivascular edema after ligation of the left carotid artery and the right jugular vein. Fixation in solution of formaldehyde, rapid trichrome stain.

Estimations of the cholesterol and the calcium in the blood were made weekly for all animals. The average initial blood cholesterol level was 135 mg per hundred cubic centimeters. During the observation period of fifteen weeks, this value gradually increased to the average maximum level of 700 mg per hundred cubic centimeters. A study of the individual responses of rabbits to cholesterol feeding was elsewhere reported. The normal blood calcium range was between 12 and 17 mg per hundred cubic centimeters, with an average value of 15.47 mg. The serum calcium was estimated by De Waard's method<sup>4</sup>. It remained within normal limits throughout the entire experiment. A drop of blood calcium in some of the rabbits given injections of epinephrine hydrochloride was not consistent and remained within normal limits. Neither the operations nor the injections influenced the chemical composition of the blood.

#### POSTMORTEM OBSERVATIONS

All organs and vessels were studied grossly and microscopically. The vessels of the neck were ex-

amined below and above the ligatures. Sections of the brains were prepared in such manner as to allow observations of all parts of the brain and all the branches of the vascular tree. Sections were stained with hematoxylin and eosin, Weigert's stain for elastic tissue, Pollak's trichrome stain and Nissl's stain for nerve cells.

Various stages of atherosclerosis were encountered: foam cell accumulation in the subendothelial layer of the intima, fibrosis of this layer, thickening of the intimal endothelium proper, hyaline changes of the fibrotic tissue, splitting of the internal elastica and secondary hypertrophy of the whole arterial wall.

In the rabbits with ligations the vessels of the neck showed dilatation and hypertrophy proximal to the ob-

<sup>5</sup> Leary, T. Arch Path **32** 507, 1941, **37** 16, 1944.

<sup>6</sup> Moon, V. H. Arch Path **3** 404 1927. Winter-nitz, M. C., Thomas, R. M., and LeCompte, P. M. The Biology of Arteriosclerosis, Springfield, Ill., Charles C. Thomas, Publisher, 1938. Page, I. H. Ann Int Med **14** 1741, 1941. Moschowitz, E. Vascular Sclerosis with Special Reference to Arteriosclerosis, New York, Oxford University Press, 1942.

<sup>4</sup> De Waard, D. J. Biochem Ztschr **97** 186, 1919.

struction and atrophy and thrombosis distal to it. Owing to this fact, there was a striking difference in type between the atherosclerosis in the carotid arteries proximal to the ligations and that above the ligatures or in

tension. In the ligated carotid arteries the process hardly advanced beyond the accumulation of foam cells and the initial thickening of the endothelium. The organization started late, after the foam cell layer spread



Fig 3—*A*, recanalization of a ligated carotid artery,  $\times 110$ . *B*, aneurysmatic bulging of the basilar artery after ligation of the left carotid artery and of the right jugular vein,  $\times 220$ . *C*, cerebral softening and beginning of formation of a glial scar after ligation of both carotid arteries,  $\times 220$ . Fixation in solution of formaldehyde, rapid trichrome stain.

any of the other vessels (fig 1 *A* and *B*). All stages of atherosclerosis were observed in untouched arteries. In these the organization of the foam cell layer started early and before the plaque reached its maximum ex-

along the whole circumference of the vessel and narrowed the lumen. The external layers of the vessel were narrow, stretched. This observation confirmed the theory that local mechanical stress or an obstacle



is an important factor in the localization and the decisive factor in the degree of the atherosclerotic lesion<sup>7</sup>

The livers and the adrenal glands of the majority of the cholesterol-fed rabbits were grossly enlarged and displayed microscopically intracellular cholesterol storage and lymphatic blockade. Beginning periportal fibrosis was visible in some slides. All cortical layers of the adrenal glands were equally involved. The kidneys of many rabbits showed microscopically chronic nephritis. Examination of the other organs did not reveal any changes.

None of the cholesterol-fed rabbits revealed any changes in the cerebral vessels which could be attributed to atherosclerosis. Rabbits with ligated carotid arteries had marked hypertrophy of all cerebral arteries, mainly of the internal elastic layer (fig 1 C and D). Sometimes there was splitting of the elastica of the basilar artery and of that of the anterior cerebral arteries, while all vessels showed thickening of this layer. In rabbits with both jugular veins ligated there were dilatation of meningeal and cerebral veins and hypertrophy of arteries. In animals in which one carotid artery and one jugular vein were tied, these reactive changes of the cerebral vessels were as consistent and always as severe as in the first two groups of animals. The small arteries of the cortex as well as of the white matter were equally involved. Dilated and thick-walled small arteries were seen in all parts of the brain (fig 2 A and B). All these abnormalities were as frequent and as extensive in the controls as in cholesterol-fed animals with analogous ligations.

Three rabbits presented unusual alterations. In only 1 animal was there new formation of capillaries in the fibrotic wall of a ligated carotid artery with reestablished circulation (fig 3 A). In another rabbit there was an aneurysmatic bulging of the dilated and thin-walled basilar artery without any signs of arteriosclerosis in this or any other vessel (fig 3 B). A third rabbit showed a microscopic area of softening with central destruction and peripheral infiltration located in the base of the right temporal lobe (fig 3 C). All these animals were fed cholesterol, and all had one or two ligated carotid arteries.

Rabbits given injections of epinephrine hydrochloride or of histamine phosphate did not show anything different from other animals of the series. The cerebral vascular changes were solely due to ligations of the large vessels of the neck. There was no difference in regard to degree or localization of atherosclerotic or other changes.

#### COMMENT

The dependence of the intravascular tension in the cerebral vessels on the systemic blood pressure has been affirmed by some authors<sup>8</sup> and denied by others<sup>9</sup> who claimed the existence of a specific control mechanism of the cerebral ves-

sels. The possibility of impairment of the cerebral blood flow by ligations of both carotid arteries or both jugular veins was the basis of the major part of the experiments reported here.

A number of experimental animals in which both carotid arteries were ligated the same day were capable of vascular compensation through dilatation of the vertebral arteries, the circle of Willis and its branches. The opening of formerly closed capillaries<sup>10</sup> prevented the maintenance of the cerebral ischemia. None of the characteristic changes of cerebral anemia were observed in sections. The vasospasm probably reduced the blood flow. Sudden stenosis of both jugular veins would increase the tension, which then would be transmitted backward through the capillary bed<sup>11</sup>. The ligation of one carotid artery and one jugular vein was meant to disturb the equilibrium of the cerebral circulation. The observations on large and small cerebral vessels after all three types of ligations proved that a disturbance occurred. The alterations were consistent with the changes described in hypertension. They were due to an impairment of the rate of the blood flow and a change of the intravascular tension.

The increase in vascular resistance was insufficient to transform the cerebral vessels into a locus minoris resistentiae for the deposit of cholesterol. To achieve this, other means should be tried, such as compression, edema, destruction of the capillary bed or diminution of the brain volume by chronic disease or by adhesive meningitis.

Epinephrine hydrochloride injected into the carotid arteries should decrease the cerebral circulation by causing contraction of the arteries. The drug would act as a direct irritant of the arteries, and the increased blood pressure would be a supporting factor. Histamine phosphate similarly injected should increase the circulation by causing relaxation of the arteries, with slowing of the blood flow and lowering of the blood pressure. Both epinephrine and histamine disturb the nourishment of the vessels by compression of the vasa vasorum and by impairment of the oxygen consumption<sup>12</sup>. More severe changes in the cerebral vessels were expected.

10 Sjostrand, T. *Skandinav Arch f Physiol* 68 160, 1934, (supp.) 71 1, 1935.

11 Roy, C. S., and Sherrington, C. S. *J Physiol* 11 85, 1890. Hill, L. *The Physiology and Pathology of the Cerebral Circulation. An Experimental Research*, London, J & A Churchill, 1896, pp 73-74.

12 Bredl, A., and Reiner, M. *Arch f d ges Physiol* 73 385, 1897, 79 158, 1900. Hueper, W. C. *Medicine* 20 397, 1941.

7 Dill, L. V., and Isenhour, C. E. *Arch Path* 33 655, 1942. Wilnes, S. L. *Am J Path* 18 63, 1942.

8 Gaertner, G., and Wagner, J. *Wien med Wchnschr* 37 601, 1887.

9 Forbes, H. S. *Arch Neurol & Psychiat* 19 751 1928. Ask-Upmark, E. *Acta psychiat et neurol*, 1935, supp 6, p 1.

from a combination of ligations and injections of these drugs

The fact that in the experiments epinephrine and histamine had no morphologically visible effect and did not enhance the changes caused by ligatures cannot be held decisive. There is a possibility that the doses were too small and the experimental period too short. Intermittent use of constricting and dilating drugs should also be tried.

#### CONCLUSIONS

Atherosclerosis generalized except for the cerebral vessels developed in cholesterol-fed

rabbits. Control animals, not fed cholesterol, did not show atherosclerosis.

Ligations of both carotid arteries, of both jugular veins or of one artery and one vein were followed by changes in the cerebral vessels in both the cholesterol-fed and the control animals. These changes were not atherosclerotic but hypertensive.

Injections of epinephrine hydrochloride and of histamine phosphate had no visible effect on the cerebral vessels nor did they alter the changes which were due to ligations of vessels. They did not influence the atherosclerotic lesions in any way.

# MELANOMA OF THE SMALL INTESTINE

PETER A HERBUT, M.D., AND WILLIS E MANGES, M.D.

PHILADELPHIA

Among the many organs and tissues of the body affected with melanoma, the small intestine has received hardly more than a passing comment, particularly in current textbooks of pathology. Thus Ewing<sup>1</sup> stated that "melanoma of the intestine, occurring almost exclusively in the rectum has been described in isolated reports by various observers." Boyd<sup>2</sup> remarked that "the pia mater, the adrenal, and the intestine, especially the rectum, may be the site of the primary tumor." Karsner<sup>3</sup> said that "melanoma has been reported as a rare occurrence in the small intestine" and that "melanotic tumors are likely to show metastases in the intestinal canal." MacCallum<sup>4</sup> dismissed the subject by saying that nevi are not essential for the starting of melanoma because "quite similar tumors" are found, among other organs, in the intestine. Finally, melanoma of the small bowel is not even mentioned in the textbooks of Bell<sup>5</sup> and Anderson<sup>6</sup>.

That the subject is of more than passing interest is attested by the increasing number of reports in recent medical literature. In a review of the latter we have been able to collect 25 pertinent cases. In 9 the melanoma of the small intestine was reported as primary<sup>7</sup> and

in 16 as metastatic.<sup>8</sup> Although we may have missed a few since some of the 25 cases were described under titles not suggesting such a tumor, the still rather scanty number recorded in the literature indicates either that many observed cases have not been reported or that involvement of the small intestine is not common. In reports on large series of cases of melanoma arising in the skin, the small intestine is infrequently mentioned. Adair,<sup>9</sup> in a study of 400 cases, said nothing of the intestine except that primary melanoma can arise in this organ. Plewes<sup>10</sup> reviewed 97 cases of melanoma and described 1 case with widespread metastasis in which there was also one nodule in the jejunum. In studies by Greenblatt, Pund and Bernard,<sup>10</sup> Daland and Holmes,<sup>11</sup> De Cholnoky,<sup>12</sup> and Howes and Birnkrant<sup>13</sup> involvement of the small intestine was not commented on.

In searching the literature under headings mentioning tumors of the small bowel we noted that there are only few remarks on melanoma. It was not mentioned in a summary of the litera-

From the Clinical Laboratories, Jefferson Medical College Hospital.

1 Ewing, J. *Neoplastic Diseases*, ed 4, Philadelphia, W. B. Saunders Company, 1940, pp 954 and 965.

2 Boyd, W. *A Text-Book of Pathology*, ed 3, Philadelphia, Lea & Febiger, 1938, pp 322-323.

3 Karsner, H. T. *Human Pathology*, ed 6, Philadelphia, J. B. Lippincott Company, 1938, p 534.

4 MacCallum, W. G. *A Textbook of Pathology*, ed 7, Philadelphia, W. B. Saunders Company, 1940, p 1101.

5 Bell, E. T. *A Text-Book of Pathology*, ed 5, Philadelphia, Lea & Febiger, 1944, p 327.

6 Anderson, W. A. D. *Synopsis of Pathology*, St Louis, C. V. Mosby Company, 1942.

7 (a) Vander Veer, E. A., and Kellert, E. *New York State J. Med.* **17** 335, 1917. (b) Cox, H. H., and Sloam, L. H. *J. A. M. A.* **82** 2021, 1924. (c) Lund, F. B. *New England J. Med.* **201** 1133, 1929. (d) Boyce, F. F., and McFetridge, E. M. *Internat. S. Digest* **17** 131, 1934. (e) Menne, F. R., and Beeman, J. A. P. *Am. J. Digest. Dis. & Nutrition* **3** 786,

1936. (f) Fische, F. A. *Ann. Surg.* **106** 221, 1936. (g) Gordon, W. C. *Rev. Gastroenterol.* **8** 36, 1941. (h) Morrison, W. A., and Donath, D. *California & West Med.* **55** 235, 1941.

8 (a) Peritz, E. *Arch. f. klin. Chir.* **139** 242, 1926. (b) Saphir, O. *Arch. Path.* **4** 22, 1927. (c) Maxwell, W. M. J. *Australia* **15** 656, 1928. (d) Robb, D. *Brit. M. J.* **2** 1007, 1929. (e) Wilbur, D. L., and Hartman, H. R. *Ann. Int. Med.* **5** 201, 1931. (f) Plewes, B. F. *Am. J. Cancer* **26** 732, 1936. (g) Mallory, T. B. *New England J. Med.* **218** 1013, 1938. (h) Maier, G. *Schweiz. Ztschr. f. allg. Path. u. Bakt.* **3** 106, 1940. (i) Mackay, F. H., and Hurteau, E. F. *Tr. Am. Neurol. A.* **67** 82, 1941. (j) Morison, J. E. *Brit. J. Surg.* **29** 139, 1941. (k) Bizzozero, O. J., and Collins, J. O. *Connecticut M. J.* **5** 193, 1941. (l) Jones, W. C., Dowlen, L. W., and Rand, F. H. *Bull. Jackson Mem. Hosp.* **4** 34, 1942. (m) Phillips, J. R. *Am. J. Digest. Dis.* **10** 147, 1943.

9 Adair, F. E. *Surg., Gynec. & Obst.* **62** 406, 1936.

10 Greenblatt, R. B., Pund, E. R., and Bernard, G. T. *South. M. J.* **29** 122, 1936.

11 Daland, E. M., and Holmes, J. A. *New England J. Med.* **220** 651, 1939.

12 De Cholnoky, T. *Ann. Surg.* **113** 392, 1941.

13 Howes, W. E., and Birnkrant, M. *Am. J. Surg.* **60** 182, 1943.

ture by Nickerson and Williams<sup>14</sup> in 1937 or in one by Frank, Miller and Bell<sup>15</sup> in 1942. Neither was melanoma mentioned in a study of 22 cases of malignant tumor of the small bowel reported by Medinger<sup>16</sup> or in 8 cases encountered in 2,252 autopsies reported by Chant<sup>17</sup>. Morrison and Donath,<sup>18</sup> however, recorded 2 cases of melanoma among 45 cases of tumor encountered in 25,621 autopsies. In reference to tumor of the small intestine causing intussusception, Fische<sup>17</sup> described a case of melanoma, but neither in Wangenstein's<sup>18</sup> book on intestinal obstructions nor in the textbooks of pathology previously referred to is melanoma included in a list of tumors responsible for this complication.

mary tumor of the small bowel or whether such a growth is in every case secondary to a primary focus elsewhere. The cases described were among 12 cases of melanoma encountered in the last 5,000 necropsies at the Jefferson Medical College Hospital.

#### REPORT OF CASES

**CASE 1**—A white man 22 years of age was admitted with complaints of cough and expectoration, pains in the head, chest and stomach, and loss of 14 pounds (6.4 Kg), all occurring in the course of one month. About a year before admission there was a gradual onset of a mental change consisting of irritability, speech difficulty and impairment of intellect. On several occasions before the onset of any symptoms he attempted to remove a mole from his chest with a

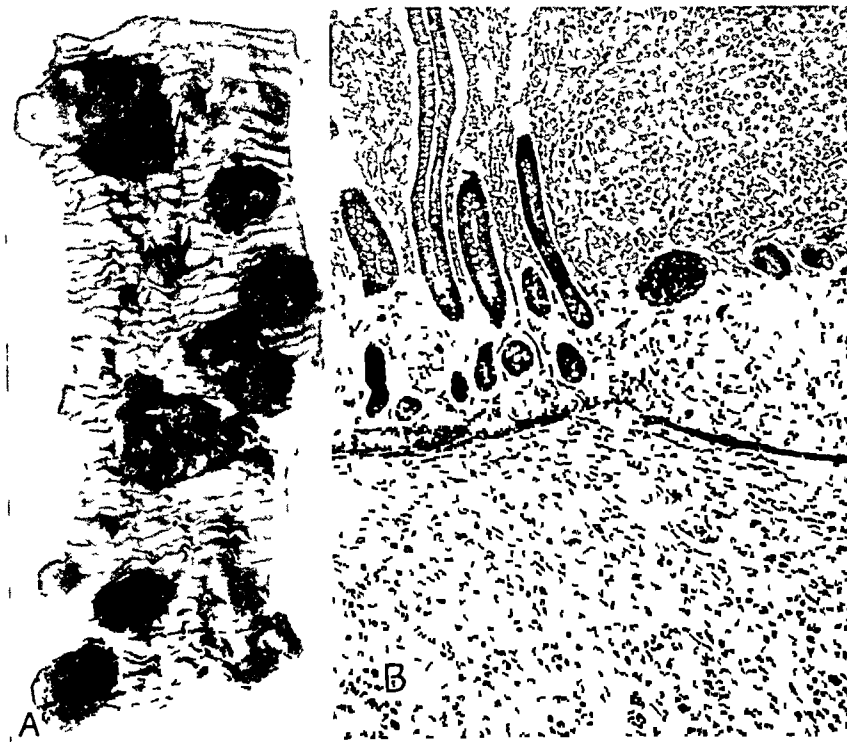


Fig 1 (case 1)—*A*, segment of the small intestine showing at least twenty-two blackish tumors of various shapes and sizes. *B*, photomicrograph of a section of the small intestine showing tumor cells diffusely infiltrating the submucosa, breaking through the submuscular mucosa and partially replacing the mucosa. Although finely granular brown pigment is abundant within the cytoplasm of the tumor cells, it cannot be seen with this magnification. Hematoxylin and eosin,  $\times 75$ .

It is the purpose of this presentation (1) to describe 5 cases of melanoma of the small intestine, (2) to draw special attention to the accompanying clinical manifestations and (3) to consider whether melanoma can arise as a pri-

mar tumor. Examination disclosed a pigmented nevus 2 cm in diameter over the right sixth rib in the midaxillary line, enlarged lymph nodes in the right axilla and bilateral papilledema. Both the mole and the axillary lymph nodes were excised for study. His condition became progressively worse, and he died one month after admission.

**Necropsy**—The body was somewhat emaciated. In the right midaxillary line the skin disclosed two recently healed surgical scars, each 5 cm long. One was at the base of the axilla and the other at the level of the sixth rib. There were no cutaneous moles or subcutaneous nodules.

In the midportion on the small intestine there was an intussusception 12 cm long. The opposing serosal surfaces were hemorrhagic, covered with fibrinous exudate and loosely adherent to each other. The bowel proximal to the invagination was dilated to a diameter

<sup>14</sup> Nickerson, D. A., and Williams, R. H. *Am J Path* **13** 53, 1937.

<sup>15</sup> Frank, L. W., Miller, A. J., and Bell, J. C. *Ann Surg* **115** 544, 1942.

<sup>16</sup> Medinger, F. G. *Surg, Gynec & Obst* **69** 299, 1939.

<sup>17</sup> Chant, L. K. *Radiology* **36** 86, 1941.

<sup>18</sup> Wangenstein, O. H. *Intestinal Obstructions*, ed 2, Springfield, Ill., Charles C Thomas, Publisher, 1942, p. 397.

of 8 cm, while distally it was completely collapsed. At the apex of the intussusception there was a polypoid tumor mass 3 cm across and 1 cm thick, which was attached to the mucosa by a pedicle 1.5 cm in diameter. In the mucosa and the submucosa of the entire small bowel both proximal and distal to the intussusception there were 150 rather firm tumor nodules measuring from 0.1 cm to 3 cm in diameter (fig 1A). Their color varied from pinkish gray to dark brown or black. Some possessed thin or broad pedicles, but others were entirely submucosal, and none penetrated the deeper layers of the bowel. Most of them were superficially ulcerated. Tumor nodules were not found in the large intestine or in the stomach.

The mesentery contained a few enlarged hemorrhagic to black nodes of cancer tissue, measuring 1.5 cm in diameter. In the pancreas there were three gray to black tumor masses measuring as much as 3 cm in diameter, and a single similar nodule was found in the

stained nuclei, and even more sparsely dispersed were a few cells containing two piled-up nuclei. Mitosis was not seen. Brown pigment was irregularly scattered throughout the cells.

Sections of the surgically removed axillary lymph nodes disclosed almost complete replacement of the nodes with tumor nodules. The smaller, apparently more recent nodules consisted of masses of anaplastic cells with either a diffuse arrangement or a tendency to whorl formation. The cells showed variation in shape and size. The cytoplasm was abundant and deep pink. The nuclei were irregular and hyperchromatic. Frequently there were large bizarre giant cells with abundant cytoplasm and single or many irregularly shaped hyperchromatic nuclei. These nodules of tumor cells were surrounded peripherally with varied amounts of fibrous tissue. There were other nodules in which the fibrous tissue was more abundant and the tumor cells fewer, and still others consisting almost entirely

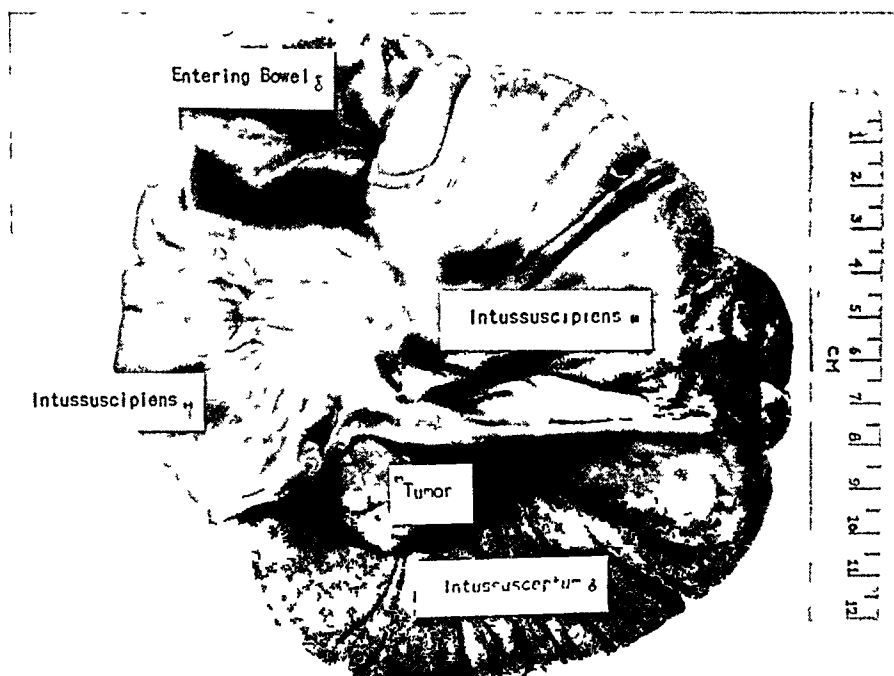


Fig 2 (case 2) —A section of the small intestine showing intussusception. At the apex of the intussusceptum there is a tumor mass.

lateral and basilar portion of the upper lobe of the right lung. The remaining thoracic and abdominal organs, including the adrenal glands and the liver, were normal.

The brain weighed, 1,560 Gm. The cerebral hemispheres were enlarged but symmetric. The medial and posterior portions of each frontal lobe contained a small bulge which on section disclosed gray to black tumor masses measuring as much as 2.5 cm in diameter. There were other smaller nodules in each cerebral hemisphere and one in the pineal gland. The eyes were not examined.

Microscopic sections of the surgically removed nevus disclosed an intact epidermis with well developed pegs and considerable pigment in the basal cell layer. The outer third of the dermis was completely infiltrated with two types of cells, arranged singly, in cords, in sheets and in a few areas in whorls. Most of the cells were uniformly stained and were regular in shape and size. They were either round with a moderate amount of pale cytoplasm and round nuclei or oval to spindle shaped with scanty cytoplasm and spindle-shaped evenly stained nuclei. Occasionally there were more deeply

of fibrous tissue. Deep brown pigment was abundant in all sections, both free and within cells.

Microscopic sections of the nodules from the lung, the pancreas, the mesenteric lymph nodes, the intestine and the brain were essentially similar, and all resembled those of the axillary nodes just described. In addition to the large cells with abundant pink cytoplasm seen almost exclusively in the axillary nodes, many of the sections obtained at autopsy showed masses of spindle cells with abundant elongated cytoplasm and hyperchromatic spindle-shaped nuclei. Brown pigment, which failed to take a stain for iron, was scanty in some areas while in others it was extreme, obliterating the underlying cells. Fibrous tissue proliferation was minimal, but capillaries engorged with erythrocytes were prominent. In the intestine, although the smaller tumors were located entirely within the submucosa, their relation to the capillaries or to the lymph channels could not be determined. The larger tumors penetrated the submuscular mucosa, infiltrated and destroyed the mucosa and gave rise to irregular ulcers on the surface (fig 1B). Sections of the bowel wall made through the intussusception showed diffuse hemorrhagic infarction

CASE 2—A 50 year old white man was admitted with the following complaints of a year's duration dull pains and noises in the abdomen, loss of appetite with spells of vomiting, constipation, sciatica, a mass in the lower part of the abdomen on the right side, and loss of about 70 pounds (32 Kg) in weight. Examination disclosed emaciation, borborygmi, a movable mass in the right side of the abdomen and a nonmovable mass over the left sixth rib anteriorly. He died two weeks after admission.

*Necropsy*—The body was poorly nourished. There were two subcutaneous tumor masses on the left side of the thorax. One measured 8 cm in diameter and was located at the level of the costochondral junction of the sixth rib, the other was 3 cm in diameter and situated at the level of the seventh rib in the midaxillary line. A third tumor mass, measuring 12 cm across, was firmly embedded in the abdominal wall of the right upper quadrant. Each mass was firm and on section light gray.

The peritoneal cavity contained about 800 cc of turbid watery exudate. About 10 cm proximal to the ileocecal valve there was an intussusception 20 cm long. The midportion of the intussusceptum contained at the attachment of the mesentery a perforation measuring 5 cm in diameter. Through this protruded a portion of the intussusceptum. It was deep red and cyanotic and had at its apex a pedunculated tumor mass 3 cm in diameter. The opposing serosal surfaces of the invaginated bowel were covered with fibrinous exudate. The entire portion of the small intestine above the intussusception was greatly distended, that below the obstruction, along with the large intestine, was completely collapsed. In the jejunum and the proximal portion of the ileum the mucosa and the submucosa presented many tumor masses varying from 1.5 to 7 cm in diameter. Some were attached by broad pedicles, others by more elongated pedicles, while still others were sessile. Some were superficially ulcerated, and on section all were pinkish gray. In the mesentery there were numerous masses, 2.5 cm in diameter, also composed of firm pinkish gray tumor tissue.

The right adrenal gland was completely replaced with a tumor measuring 15 cm by 10 cm. It was well encapsulated and easily separated from the liver, the inferior vena cava and the kidney. The cut surface disclosed an external rim of firm dark gray tissue 2 cm thick, enclosing a central mass of friable reddish brown tissue. One pole of the left adrenal gland contained a nodule of gray tumor tissue 1.5 cm in diameter. Similar nodules were found in the kidneys, the lungs and the body of the fifth lumbar vertebra. Permission to examine the head was not granted.

Many sections from the tumor masses described showed a similar histologic picture. The cells were arranged diffusely, in strands, in whorls and occasionally around blood capillaries. They varied remarkably in shape and size. While most were polygonal, a few were definitely elongated or spindle shaped, and there were many grades between these two extremes. The cell borders were distinct, and the cytoplasm was always stained deep pink and abundant. The nuclei were of all shapes and sizes, but most often they were large. They showed marked hyperchromatism and only scattered mitoses. Throughout the sections were varied numbers of giant cells with abundant cytoplasm and several piled up, deeply stained nuclei. Brown pigment was seen in only a few cells. Capillaries were not abundant. Frequently in the larger tumors there were extensive areas of necrosis. In the intestine the smaller nodules

were in the submucosa, but as they increased in size both the mucosa and the muscle coats were infiltrated. In sections of the adrenal glands the tumor cells infiltrated between the cortical cells, but no transition from one to the other could be demonstrated.

CASE 3—A white man 63 years old stated that eleven months previously a mole was removed from the right side of his neck because it bled occasionally and was increasing in size. Two months before admission "moles" began to appear on the face, the neck, the hands and the legs, and about the same time he began to have increasing epigastric pain. Examination showed many subcutaneous nodules over the entire body and an enlarged liver. He died three weeks after admission.

*Necropsy*—The body was somewhat emaciated. Throughout the entire subcutaneous tissue there were numerous tumor nodules measuring as much as 3 cm in diameter. Some were round, others were flat, and their color varied from brown to black. The mucosa and the submucosa of the entire small intestine contained numerous black tumor nodules varying in size from that of a pinhead to 0.5 cm. None was found in the esophagus, the stomach or the large intestine. Each adrenal gland weighed 40 Gm and measured 7 by 4.5 by 1.5 cm. Sections disclosed them completely replaced with soft brown to black tumor tissue. Black circumscribed tumors measuring as much as 3 cm across were also present in the mesenteric lymph nodes, the omentum, the peritoneum, the pancreas, the liver, the kidneys, the heart, the base of the left lung and the left testicle. There was no metastasis to the brain or the liver.

Microscopic sections of the tumors disclosed a uniform picture. In most of the sections deep brown pigment was so abundant that the underlying neoplastic cells could scarcely be seen. In areas where the pigment was less abundant the cells were arranged in sheets or cords or more diffusely. They were either round or polygonal and rather uniform in size. The cell outlines were not always distinct, but the cytoplasm was stained deep pink and often was filled with brown granules. The nuclei were round, evenly stained and uniform. There were no giant cells. In the intestine, as in the preceding cases, the tumors were primarily in the submucosa, but as their size increased both the mucosa and the muscle coats were invaded. The relationship of the submucosal blood vessels and lacteals to the tumors could not be determined from a study of the available sections.

CASE 4—A white man 67 years old said that two years previously a pimple on his right cheek was squeezed and subsequently repeatedly irritated while shaving. It steadily increased in size. In the past few months he noticed lumps appearing over his body, began to have pains in the joints and lost about 40 pounds (18 Kg) in weight. Years ago as a result of a trauma he lost an eye, and thirty years previously he had a mixed tumor of the right parotid gland which was removed surgically. Examination disclosed an ulcerating tumor of the right cheek, measuring 3 cm in diameter, many subcutaneous nodules scattered over the entire body, and a swelling of the lower end of the right femur. He died a month after admission.

*Necropsy*—There was a foul-smelling ulcerated gray to black tumor in the skin of the right cheek, measuring 2.5 by 1 cm. There were numerous nonulcerated tumor nodules in the subcutaneous tissue of the entire body measuring from 0.3 to 5.0 cm in diameter. They were sharply circumscribed, freely movable, gray to black, and firm. There were five tumor masses in the mucosa

and the submucosa of the stomach, one in the duodenum, thirty-three in the small intestine and five in the large intestine. They measured from 0.2 cm to 5.0 cm in diameter and were pedunculated or sessile. Their apices were convex or concave, and many showed superficial ulcerations. While most were limited to the mucosa and the submucosa, a few penetrated to the serosa. They were firm and on section pinkish gray to black. In the midportion of the small intestine there was an agonal intussusception, which disclosed a tumor at the apex of the invaginating bowel. The right adrenal gland measured 5 by 3 cm and was completely replaced with tumor tissue. The left one was normal. There was no metastasis to the liver, but tumors were present in the lymph nodes of the mesentery, along the abdominal aorta and the mediastinum, in the myocardium, the peritoneum, the pancreas, the right kidney, the right clavicle, the lower end of the right femur and the brain.

Microscopic sections of a portion of the ulcerating tumor of the right cheek, removed surgically, together with many sections of the tumors removed at autopsy, showed a similar structure. The cells were arranged diffusely. They were round, oval or polygonal and contained an abundant amount of pink-staining cytoplasm. The nuclei varied greatly in shape and size and showed marked hyperchromatism but no mitosis. There were scattered giant cells, each with several indistinct nuclei. The central portions of the larger neoplastic masses showed considerable necrosis. Brown pigment, both free and within cells, was scanty, but the dopa reaction on a portion of the tumor removed surgically from the right cheek was strongly positive.

**CASE 5**—A 38 year old white man was admitted with the following symptoms, which started nine months previously: multiple nodules in the skin, pain in the epigastrium, occasional vomiting, constipation, loss of appetite, weakness, and loss of 33 pounds (15 Kg) in weight. Examination disclosed numerous nodules in the skin over the head, the chest and the abdomen and an enlarged nodular liver. Bouts of vomiting and epigastric pain became increasingly severe until he died two months later.

**Necropsy**—In the skin and the subcutaneous tissues of the entire body there were numerous nodules varying from pinpoint size to 2 cm in diameter. They were firm, sharply circumscribed, not ulcerated and on section gray except for a few that were distinctly brown. In the mucosa and the submucosa of the duodenum there were a "number" of gray firm tumor nodules 0.1 to 0.2 cm in diameter. The rest of the gastrointestinal tract was normal. Each adrenal gland was "enlarged" and on section disclosed within its substance numerous gray circumscribed tumor nodules. Similar nodules, sometimes as much as 3 cm in diameter, were found in the peritoneum, the pericardium, the myocardium, the lungs, the kidneys and the liver. There were no pigmented moles or scars in the skin and no tumors within the brain.

Microscopic sections from all areas showed similar tumor tissue. The cells were diffusely arranged and were either relatively small, with indistinct cytoplasm and spindle-shaped nuclei, or large and polyhedral, with abundant, sharply circumscribed, deep pink-staining cytoplasm and large, round or irregular hyperchromatic nuclei. The larger cells frequently contained vacuoles. Mitosis was not seen. Brown pigment was usually scanty or absent, but in some of the nodules from the peritoneum it was abundant. Sections of the duodenum disclosed tumor nodules within both the submucosa and the mucosa, causing ulceration of the latter

in several areas. In the adrenal gland tumor cells replaced the medulla and were also insinuated between the cells of the cortex. In some sections there appeared to be a gradation from normal adrenal tissue to tumor tissue, but usually the line of demarcation was distinct.

#### COMMENT

Clinically, melanoma of the small intestine is frequently accompanied by abdominal pain with or without other symptoms of intestinal obstruction. In 14 of the 25 cases recorded in the literature there were varied combinations of pain and fulness in the epigastrium, nausea and vomiting, anorexia, constipation or diarrhea, tarry stools, distention and tenderness of the abdomen and increased peristalsis. These were also the signs and symptoms presented in the 5 cases reported here. Sometimes, as in case 5, they may be attributed to tumor involvement of the liver, but in cases in which the liver shows no metastasis (for example, cases 1, 2, 3 and 4), they are traceable to the intestine. This is particularly true in cases in which intussusception develops, as in cases 1 and 2 of this report, and in 8 of the 25 cases collected from the literature.

Sometimes sudden colicky abdominal pain followed by nausea, vomiting and constipation is the first and only indication that something is amiss and requires prompt operation. The immediate prognosis is not always entirely hopeless because, although in the cases reported here the intestinal tumors were multiple and there were other evident metastases, in some of the cases reviewed there were only one or two tumors in the small bowel. There is a total of 4 cases in which the patient was said to have recovered after operation. One of these was reported by Vander Veer and Kellert<sup>7a</sup>. An intussusception was reduced and a solitary tumor of the ileum resected. The patient lived comfortably for six months and then died of a metastasis in the lungs. A second case was reported by Maxwell<sup>8c</sup>. The patient was operated on first for obstruction. One tumor was removed at that time. One and one half months later the patient was operated on for intussusception, and another tumor was removed. Six months later, at the time of writing, the patient was still well. A third case was reported by Lund<sup>7c</sup>. The patient had two intussusceptions caused by two tumors. Each was resected, and the patient recovered but died six weeks after leaving the hospital. A fourth case was reported by Jones, Dowlen and Rand<sup>81</sup>. The patient was operated on for obstruction and recovered. Nothing was said of his ultimate condition.



It is generally agreed that primary melanoma usually arises in the skin or the eye. Whether it can occur as a primary tumor in the small intestine has not yet been proved, in spite of occasional reports to the contrary. There are several reasons for doubting an origin in this organ. First, it has been shown by Laidlaw<sup>19</sup> and others that melanoblasts are found only in the skin and in mucous membranes of ectodermal origin. They are not found in the large intestine above the mucocutaneous junction of the rectum, and as far as we know, they have likewise not been demonstrated in the small intestine. Recently we used the dopa test on sections of duodenum, jejunum and ileum obtained from a man one-half hour after his death. No cells in any of these sections gave a positive reaction. Simultaneously tested sections of the skin, however, reacted strongly. Therefore, in the absence of melanoblasts, a melanoma cannot arise in the small intestine as a primary tumor. Second, the involvement of the small intestine is exactly the same in cases in which the primary tumor has been located elsewhere as in those in which such a tumor has not been found and in which the tumor in this organ is considered primary. Third, one must always bear in mind that in cases of melanoma delayed metastatic growths, often appearing years after a "mole" has been removed, are common,<sup>20</sup> and so the primary source may be easily overlooked. Fourth, it is known that moles which grossly and histologically appear benign are sometimes known to produce metastases.<sup>1</sup> This is well illustrated in the first case reported here. Histologically, the cells were so regular that the mole was first diagnosed as a "benign" pigmented nevus. It was not until later when an examination of the draining lymph nodes disclosed metastatic melanoma, that several sections of the original mole disclosed a slight variation in the intensity of staining of some of the nuclei and an occasional cell with two nuclei. These changes were so slight, however, that if we were confronted with a similar section we should probably make the same diagnosis. Thus, since most people have one or more nevi, one cannot be sure in the cases in question that the primary site was not in a nevus of innocent appearance. Fifth, however carefully autopsies are made, they

are often of necessity not thorough enough. Although it is known that melanoma frequently arises as a primary tumor of the eye,<sup>2</sup> only in two reports<sup>21</sup> of the cases reviewed is it stated that the eyes were examined post mortem. In one other<sup>7c</sup> they were said to have been examined with an ophthalmoscope during life. Without a thorough examination of the eyes, a diagnosis of primary melanoma of the small intestine should not be made. Another possible primary source that has not been sufficiently considered is in the mongol cells (melanoblasts) which in infants of all races lie deep in the corium over the sacrum and along the back. According to Laidlaw,<sup>19</sup> these are true melanoblasts and are therefore capable of giving rise to a melanoma. Since these cells are present in the newborn, some will undoubtedly persist throughout adult life and may be the starting point of melanoma. This may be particularly true in those cases in which multiple subcutaneous nodules occur over the entire body, including the back, with no demonstrable primary site in the usual locations.

#### SUMMARY

A review of the literature discloses reports of approximately 25 cases of melanoma of the small intestine. In 9 of these the tumor was reported as primary in the small bowel, in 16, as metastatic. To the latter group we have added 5 cases. In 3 of these the neoplasm originated in a cutaneous nevus, while in 2 the primary site was not determined. Abdominal pain, with frequently other symptoms of intestinal obstruction, were present in 18 of the 30 cases now reported, while intussusception occurred in 10. It appears that melanoma of the small bowel is usually, if not always, a metastatic tumor for the following reasons: (1) Melanoblasts have not been demonstrated in the small intestine, (2) the involvement of the bowel in secondary melanoma is the same as that in the cases in which the tumor of the bowel is thought to be primary, (3) primary cutaneous growths may be lost sight of in the often delayed metastasis peculiar to melanoma, (4) occasionally quiescent moles are known to give rise to local and distant metastases, and (5) autopsies are often albeit of necessity, not thorough enough.

<sup>19</sup> Laidlaw, G. F. *Am J Path* 8:477, 1932.

<sup>20</sup> Wilbur and Hartman<sup>8c</sup> Bizzozero and Collins<sup>8k</sup>

<sup>21</sup> Fische<sup>7f</sup> Peritz<sup>8i</sup>



# CHEMICAL BASIS OF FEVER WITH INFLAMMATION

VALY MENKIN, M D

DURHAM, N C

Recent studies undertaken by me have demonstrated in exudate the presence of a substance which offers a reasonable explanation for the basic pattern of injury in inflammation<sup>1</sup>. This substance, which either is the euglobulin fraction of exudate or at least is associated with it, has been termed necrosin. It is presumably liberated from cells which have been initially injured by an irritant. The internal chemical constitution of the damaged cell is doubtless altered, yielding as a result various common factors, which in turn are responsible for the unfolding of the fundamentally stereotyped pattern of inflammation. Leukotaxine, the leukocytosis-promoting factor, and necrosin belong to such a category of chemical units formed by injured cells. In this connection necrosin has been found to induce a severe inflammatory reaction, accompanied by a lymphatic blockade<sup>1</sup>. When it is introduced into the circulating blood leukopenia follows promptly and is replaced several hours later by leukocytosis<sup>1b</sup>. The internal organs are injured, notably the liver and to some extent the kidneys<sup>1b</sup>. Besides fatty deposits in the parenchyma, small foci of leukocytic infiltration may be found irregularly scattered throughout these organs<sup>1b</sup>.

Further studies on dogs have indicated that the intravascular injection of necrosin is accompanied by a rapid elevation in temperature<sup>2</sup>. This hyperthermia was not induced by other protein fractions derived from exudate, ascitic fluid or normal blood serum.

Inasmuch as necrosin seems to penetrate into the circulating blood<sup>1b</sup> from the site of inflammation it is conceivable that the fever produced with inflammation may be referable in large part

to the action of the necrosin absorbed from the site of injury into the blood stream. The present communication presents further data on the active chemical substance involved in the development of fever. There is present in exudate a pyrogenic factor, termed for convenience pyrexin, which seems to be formed by the enzymatic action of necrosin, which is in turn closely associated with the euglobulin fraction of exudate.

The early writers have demonstrated that with various infectious processes accompanied by fever there is manifest an increase in protein metabolism<sup>3</sup>. Furthermore, this enhancement seems to be wholly independent of the low level of the normal "wear and tear" of nitrogenous metabolism<sup>4</sup>. These facts have led to the conclusion that fever involves severe toxic destruction of proteins<sup>5</sup>. Coleman and Dubois<sup>6</sup> in an exhaustive study inferred that the destruction of proteins in typhoid is referable to the toxins of that disease. Leyden and Klempeier<sup>7</sup> reported that in the high fever of pneumonia nitrogenous equilibrium cannot be attained. The output of nitrogen in the urine is high, and this state of affairs continues after the crisis until the excess nitrogen derived from the exudate is eliminated. It is of interest to note that patients who have succumbed in high fever show on autopsy parenchymatous and fatty degeneration of some of the internal organs<sup>8</sup>. Welch<sup>9</sup> noted similar effects in rabbits exposed to high temperature for a week. As stated in the opening paragraph, necrosin induces fatty degenerative changes in the liver and the kidneys<sup>1b</sup>.

Krehl and Matthes<sup>10</sup> reported an increase in albumoses in the urine of patients with fever

This paper is no. 31 in a series entitled "Studies on Inflammation."

This study was aided by grants from the Johnson and Johnson Research Foundation, New Brunswick, N J, and from the Dazian Foundation for Medical Research.

From the Fearing Research Laboratory, Free Hospital for Women, Brookline, Mass. Present address of author: Department of Pathology, Duke University School of Medicine, Durham, N C.

1 Menkin, V (a) *Science* **97** 165, 1943, (b) *Arch Path* **36** 269, 1943.

2 Menkin, V *Proc. Soc. Exper. Biol. & Med.* **54** 184, 1943.

3 Pfleger, E. *Arch. f. d. ges. Physiol.* **18** 247, 1878.

4 Kocher, R. A. *Deutsches Arch. f. klin. Med.* **115** 82, 1914.

5 Muller, F., cited by Lusk,<sup>8</sup> p. 513.

6 Coleman, W., and Dubois, E. F. *Arch. Int. Med.* **15** 887, 1915.

7 Leyden and Klempeier, cited by Lusk,<sup>8</sup> p. 520.

8 Lusk, G. *The Elements of the Science of Nutrition*, Philadelphia, W. B. Saunders Company, 1923.

9 Welch, W. H. *M. News, Philadelphia* **52** 393, 1888.

10 Krehl, L., and Matthes, M. *Deutsches Arch. f. klin. Med.* **54** 501, 1895.

These substances were found toxic in animals Klempeier<sup>11</sup> insisted that the toxicity was referable to impurities, and not to the albumoses of the urine Mandel<sup>12</sup> observed an increase in purine bases in the urine of febrile patients fed with milk In the opinion of this author the purine bases liberated by destruction of tissue may be of considerable significance in inducing fever<sup>13</sup> Erben reported an increase in xanthine bases and amino acids in measles and chicken-pox<sup>14</sup> Xanthine bases were found also somewhat increased in scarlet fever and typhoid Senator<sup>15</sup> and Mendelson<sup>16</sup> showed that in a dog an injection of pus is followed by an elevation in temperature Lusk<sup>8</sup> pointed out that the rise in temperature induced by experimentally puncturing the neurogenic heat centers may have little to do with the true mechanism of the production of fever in infections Barbour discussed the various views on the possibility that the heat centers are "stimulated" in fever<sup>17</sup> Grafe<sup>18</sup> referred the production of fever to the irritating action on the heat centers of the products of decomposition of bacteria or of injured tissues

#### EXPERIMENTAL STUDIES

In a series of experiments an attempt has been made to determine with greater exactitude the component which is responsible for the elevation in temperature following the intravascular injection of the euglobulin fraction of exudate

The studies have all been made on rabbits This animal was found to be convenient and far simpler to handle than the dog A preliminary note on these earlier observations has recently appeared<sup>19</sup> Various fractions of exudate and of necrosin were injected into the circulating blood stream, and the rectal temperature was recorded at intervals during a period of several hours

Necrosin was usually prepared from canine exudative material as described in a previous communication<sup>1b</sup> An acute pleural inflammation was induced by injecting about 1.5 cc of turpentine<sup>1b</sup> In few instances material obtained from human exudates was employed The various tests made and the further fractionation of necrosin will be described as the observations and the results obtained are being presented

*Variation in Temperature in Normal Rabbits*—It is important to determine first of all the daily fluctuation of temperature in rabbits The animals during a period

of six to seven hours do not show much variation The average maximum rise in temperature during such an interval is less than 1 F (0.63 F, table 1 and chart 1) From day to day, however, the rabbit may display temperature fluctuation, but this fails to occur to any appreciable extent within the duration of an experiment that lasts six to seven hours It should perhaps be pointed out that the rabbit may display relative instability during excessively warm days Note in table 1, for instance, that although the temperature of rabbit 23-55 normally fails to fluctuate, on a very warm day this rabbit showed a maximum increase of 1.45 F Nevertheless, the latter is approximately the greatest rise in temperature referable solely to weather This increase is, however, definitely transcended when a potent pyrogenic derivative associated with the euglobulin fraction of exudate is injected

TABLE 1—*Variation in Temperature in Normal Rabbits and in Animals into Which Inert Materials Were Injected*

Rabbit Number	Maximal Increase in Temperature Over a Period of About 6 to 7 Hr.		
	Basal Temperature, F	Temperature, F	Temperature, F
23 18 normal	102.7	103.65	0.95
23 65 normal	104.75	105.3	0.55
23 55 normal	103.3	103.3	0.0
23 12 normal	102.6	103.0	0.4
23 53 normal	104.6	105.05	0.45
23 55 normal	104.3	105.75	1.45*
Average increase in temperature			0.63
23 24.2 5 cc saline solution	103.1	102.4	0.0
23 18 0.5 cc saline solution	103.4	104.25	0.85
23 15 1 cc euglobulin suspension of canine serum	102.1	102.8	0.7
23 42 1 cc euglobulin suspension of human serum	103.9	104.0	0.1
23 27 8 mg LPF† in 1 cc saline solution	103.6	104.6	1.0†
Y 0.5 cc LPF	104.1	105.0	0.9
23 19 1 cc LPF	102.9	103.0	0.1
22 92 0.5 cc albumin from canine exudate	103.0	104.35	1.35
23 27 1 cc albumin from canine exudate	102.8	103.75	0.95
Average increase in temperature			0.66

\* Excessively warm day

† LPF stands for leukocytosis promoting factor

‡ There is evidence that this fraction of LPF contained a trace of necrosin as an impurity

Studies have also been made following injections of isotonic solution of sodium chloride, of the euglobulin fraction of normal canine serum, of the euglobulin fraction of normal human serum, of the pseudoglobulin or leukocytosis-promoting factor from canine exudate and, finally, of the albumin fraction from the latter The results are summarized in table 1 Note that not one of these substances induces any appreciable rise in temperature, the average being of about the same order as that observed in normal animals, namely 0.66 F (table 1 and chart 1)

*Pyrogenic Capacity of Whole Exudate*—When 0.5 cc (or even less) of exudative canine material is injected into the marginal vein of a rabbit's ear, there ensues within a period of about one hour or less an appreciable rise in the animal's temperature The data of such experiments are recorded in table 2 (compare also chart 1) The average rise in temperature during a period ranging from about six to seven hours is 2.37 F This represents an increase of 276 per cent over the maximum rise resulting from normal variation of temperature in rabbits (table 1) The magnitude of rise in temperature does not seem to depend primarily

11 Klemperer, cited by Lusk,<sup>8</sup> p 523

12 Mandel, A R Am J Physiol **10** 452, 1904

13 Mandel, A R Am J Physiol **20** 439, 1907

14 Erben, F Ztschr f Heilk **25** 33, 1904

15 Senator, H Untersuchungen uber die fieberhaften Process und seine Behandlung, Berlin, A Hirschwald, 1873

16 Mendelson, W Virchows Arch f path Anat **100** 274, 1885

17 Barbour, H G Physiol Rev **1** 295, 1921

18 Grafe, E Deutsches Arch f klin Med **101** 209, 1910

19 Menkin, V Federation Proc **3** 32, 1944

on the amount of material injected, i. e. within limits. It has been found that at times 0.1 or 0.2 cc of exudative fluid will induce as great an effect as the injection of 0.5 cc or even more. The effect thus displays a relative "all or none" response. This seems to hold true also, to some extent, with the fraction of exudative material. These facts suggest the presence of a pyrogenic factor in inflammatory exudate.

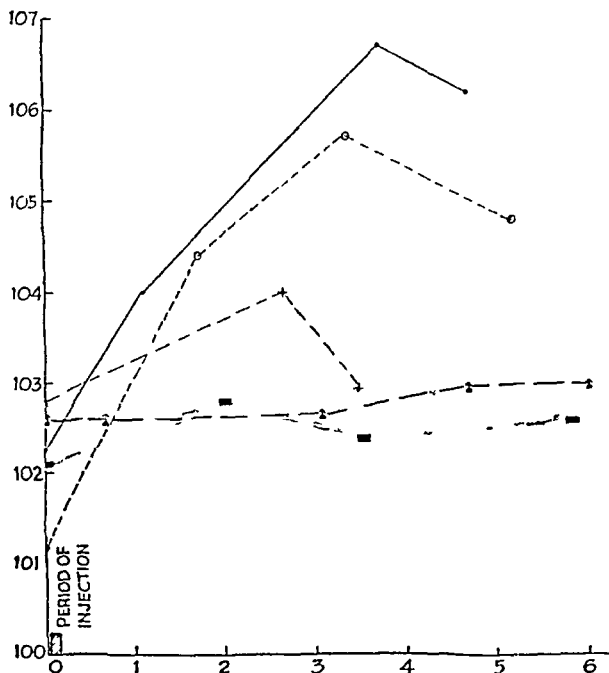


Chart 1—Effect of exudate and of other fractions of canine body fluids on the level of temperature in the rabbit, average variation of temperature in normal rabbits (—•—), temperature curve with exudate (---•---), curve with the euglobulin fraction of exudate (necrosin + pyrexin) (—+—), curve with nonhemolyzed blood serum (—■—), curve with the euglobulin fraction of normal blood serum (—△—). Degrees Fahrenheit are shown at the left, lapse of time in hours, at the bottom.

TABLE 2—Effect of Whole Exudate on Level of Temperature in Rabbits

Rabbit Number	Basal Temperature, F	Maximal Temperature Over a Period of About 6 to 7 Hr., F	Increase in Temperature, F
23-52	102.2	104.1	1.9
23-52	102.6	104.4	1.8
23-44	101.2	105.7	4.5
23-61	102.4	104.4	2.0
23-11	103.5	105.2	1.7
23-27	102.05	104.0	1.95
23-42	102.0	105.1	3.1
23-27	103.3	105.6	2.3
23-70	102.7	104.5	1.8
23-58	102.3	104.3	2.0
23-11	102.8	105.8	3.0
23-27	102.4	104.75	2.35
Average increase in temperature			2.37

*Presence of the Pyrogenic Factor in the Euglobulin Fraction of Exudate*—As indicated in table 1, neither the pseudoglobulin fraction (i. e., the leukocytosis-promoting factor) nor the albumin fraction of exudate induces any appreciable enhancement in temperature. On the other hand, the euglobulin fraction of exudative material induces a marked rise (table 3 and chart 1).

The average increase during a period of about six hours following an injection of that particular fraction of the exudate is 2.46 F. This represents a rise of about 290 per cent over that occurring in normal rabbits (table 1). This type of effect holds true as well when a series of rabbits treated with an identical euglobulin fraction derived from one sample of exudative material is studied. In 3 rabbits (23-15, 23-26 and 23-27) the level of temperature was not appreciably altered following the injection. The euglobulin fraction injected into these 3 rabbits was derived from exudate which never contained a potent necrosin but at the same

TABLE 3—Effect of Necrosin (Whole Euglobulin Fraction of Exudate) on Level of Temperature

Rabbit Number	Basal Temperature, F	Maximal Temperature About 6 Hr After Injection of Necrosin, F	Increase in Temperature, F
23-24	102.2	106.4	4.2
23-06	103.2	106.0	2.8
22-81	101.6	103.0	1.4
15-21	103.0	105.7	2.7
23-15*	102.6	105.2	2.6
23-15†	102.6	103.7	1.1
23-01 (subcutaneous injection)	102.3	104.8	2.5
23-26†	102.65	104.55	1.9
23-27†	101.85	103.3	1.45
23-27	102.25	106.7	4.45
23-27	102.2	104.2	2.0
Average increase in temperature			2.46

\* Human necrosin was injected, in all other experiments canine material was utilized.

† The animal from which this fraction of necrosin was derived had leukocytosis and had a powerful concentration of leukocytosis promoting factor in its exudate in contrast to a poor yield of necrosin. Correspondingly, the animal appeared clinically to be very well.

time contained considerable leukocytosis-promoting factor. The dog from which this exudate was obtained appeared correspondingly to be well. In general, the clinical well-being of a dog seems inversely related to the amount of toxic euglobulin or necrosin recovered from its exudate, while, on the contrary, the concen-

TABLE 4—Effect of Nonhemolyzed Blood Serum from Normal Dogs on Level of Temperature in Rabbits

Rabbit Number	Basal Temperature, F	Maximal Temperature Over a Period of About 6 to 7 Hr., F	Increase in Temperature, F
23-11	104.0	105.2	1.2
23-55	103.4	104.6	1.2
23-18	102.8	104.0	1.2
23-50	102.65	103.7	1.05
23-72	103.8	104.3	0.5
23-70	102.9	104.2	1.3
23-58	104.2	105.4	1.2
23-64	103.3	104.1	0.8
Average increase in temperature			1.05

tration of leukocytosis-promoting factor in the exudate seems to bear a direct relation to the clinical status. These observations on the pyrogenic effect of exudate and of the euglobulin fraction therefrom in rabbits confirm earlier studies on dogs.<sup>2</sup>

*Effect of Blood Serum on the Level of Temperature*—In order to control the effect obtained with exudative material, nonhemolyzed canine blood serum was injected intravenously into rabbits. The data are con-

veniently collected in table 4, and an individual experiment is illustrated in chart 1. The average increase in temperature in eight experiments in which 0.5 cc of serum was injected was 1.05 F. This constitutes an increase of 67 per cent over the normal variation (table 1). This rise is relatively inappreciable when compared with the increases of 276 and 290 per cent induced by whole exudate and the active euglobulin fraction of exudative material.

On the other hand, when hemolyzed blood serum is utilized there seems to follow a moderate increase in the level of temperature, averaging almost 2 F (table 5). This represents an increase of 209.5 per cent

TABLE 5—Effect of Hemolyzed Serum from Normal Dogs on Level of Temperature in Rabbits

Rabbit Number	Basal Temperature, F	Maximal Temperature Over a Period of About 5 to 7 Hr., F	Increase in Temperature, F
X	103.0	105.05	2.05
23 18	103.05	106.4	3.35
23 27	103.1	104.8	1.7
23 27	102.1	104.0	1.9
23 27 *	102.45	104.2	1.75
23 11 *	103.7	105.0	1.3
23 54	103.8	105.25	1.45
X	102.8	104.9	2.1
23 27	103.2	105.2	2.0
Average increase in temperature			1.95

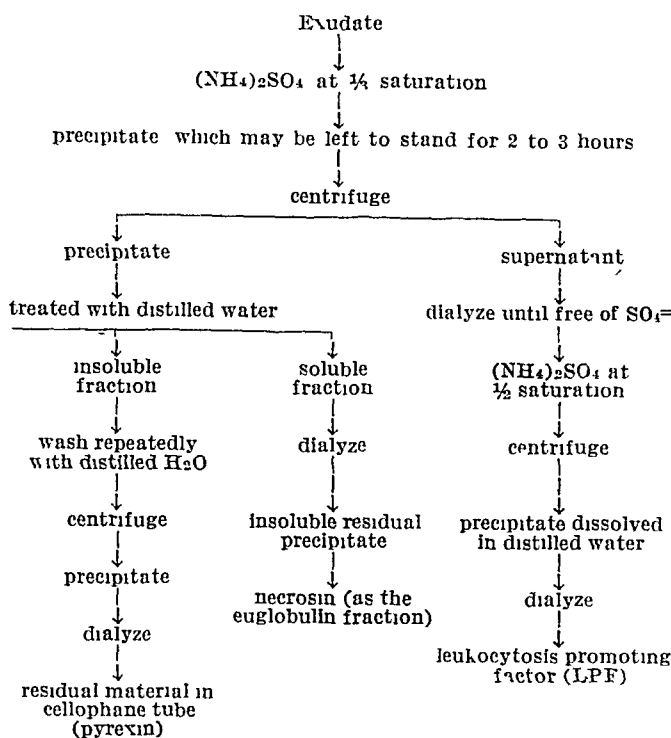
\* This animal received 0.25 cc of hemolyzed serum intravenously, 0.5 cc was injected into other animals

(table 1). These observations, in comparison with the effect of nonhemolyzed blood serum, definitely suggest that injured red cells are capable of liberating an appreciable amount of pyrogenic factor into the blood serum. This fact may prove to be of significance in helping to explain in part the fever occurring in malaria following the release of the parasites from the red corpuscles. Conceivably the pyrogenic factor may likewise be liberated alongside those organisms into the circulation. Presumably the material can probably be recovered from any injured cells, such as are obtained by crushing or by severe trauma. Such studies are being undertaken.

In a previous study<sup>1b</sup> I pointed out that necrosin, the toxic euglobulin fraction derived from exudate, was present in the blood serum only if there was concomitant inflammation. These observations suggested that necrosin penetrates from the site of injury into the circulating blood stream.<sup>1b</sup> For this reason it became of interest to determine whether nonhemolyzed serum from a dog with concomitant pleural inflammation possesses appreciable pyrogenic activity. Such serums obtained from animals with pleurisy of duration ranging from about seventeen hours to one week displayed pyrogenic potency. The evidence indicates that in at least half of the cases studied the serums were active. The average increase in temperature in such a series was 1.67 F, or a rise of 165 per cent above normal variation (table 1). When this increase is compared with the average increase noted with serums from normal dogs (table 4), a small but definite enhancement of the pyrogenic capacity of serum from animals having a concomitant inflammation is indicated. These observations are probably of significance in helping to explain the fever that frequently develops when there is an inflamed area. The pyrogenic factor absorbed into the circulation from such an acutely injured site would be capable of inducing fever. This phase of the problem is being studied further.

*Dissociation of the Euglobulin Fraction of Exudate into a Toxic Factor (Necrosin) and a Pyrogenic Factor (Pyrexin)*—The evidence described in the foregoing sections and also in previous contributions<sup>20</sup> indicates the presence of a pyrogenic factor in the euglobulin fraction of exudate. Earlier work has likewise demonstrated the toxicity of this fraction which for convenience has been termed necrosin.<sup>1</sup> Is the pyrogenic factor of exudate merely associated with necrosin or is the pyrogenic activity a property of that toxic euglobulin fraction? Studies indicate that one is dealing with two different substances which, however, seem to be closely associated as concerns their respective genesis. Recent evidence indicates that the pyrogenic factor can frequently be formed from necrosin merely by incubation of the latter. This may perhaps be brought about by enzymatic activity present in turn in the purified necrosin-containing material. The results obtained by incubation suggest this possibility. Furthermore, recent preliminary studies seem to indicate the presence of proteolytic activity in necrosin.<sup>21</sup> Fibrinogen combined with thrombin induces rapid formation of a clot which, however, with the addition of purified necrosin tends toward early liquefaction. These facts suggest either that necrosin is a proteolytic enzyme or that in its present state of purification it is closely associated with such an enzyme. On the other hand, the pyrogenic factor fails to display any such proteolytic activity.

The scheme adopted to dissociate necrosin from the pyrogenic factor or, as it is termed, pyrexin, is probably



best described by the accompanying diagram, which incidentally includes the simultaneous preparation of the leukocytosis-promoting factor.

The principle adopted in this dissociation and purification lies in the finding recently described<sup>19</sup> that the euglobulin fraction of exudate seems to be insoluble in the presence of certain electrolytes. This was at first, surmised to be due to the presence of an atypical euglobulin.<sup>19</sup> Advantage has been taken of this property in order to extract a true euglobulin from exudate. This

20 Menkin (footnotes 1b and 2)

21 Menkin, V. Am J M Sc 208 290, 1944, Science 100 337, 1944

is done by treating with distilled water in the presence of  $\text{SO}_4^{=}$  ions the precipitate formed by the interaction of the exudate with ammonium sulfate ( $[(\text{NH}_4)_2\text{SO}_4]$  at one-third saturation. In other words, a true euglobulin, by virtue of its solubility in the presence of electrolytes, is removed prior to dialysis of the  $\text{SO}_4^{=}$  ions. By this simple step the toxic material, or necrosin, is found to be either a true euglobulin or at least a substance that is associated with this fraction. In the presence of  $\text{SO}_4^{=}$  ions necrosin readily enters into aqueous solution, while, on the other hand, the pyrogenic factor seems to be primarily located in the relatively insoluble aqueous fraction. The so-called atypical euglobulin fraction of exudate represents essentially an insoluble fraction in close association with an otherwise true euglobulin.<sup>22</sup>

In brief, with the adoption of the aforementioned scheme of purification it was found that the toxic material, or necrosin, is primarily located in the true euglobulin fraction, which is essentially nonpyrogenic, while the fever-inducing property seems chiefly concentrated in an insoluble precipitate obtained as shown in the diagram.

The results of experiments with the two fractions of substances thus obtained are shown in table 6. Both of these fractions can readily be dried by freezing. It is clear that necrosin in contrast with pyrexin is essentially nonpyrogenic. The latter induces an average increase of 2.46 F. This effect is of the same order of magnitude as that obtained with the whole exudate. The latter causes a rise of 2.25 F, while purified necrosin induces an average increase of 0.94 F. The

factor in exudate that is responsible for the potent pyrogenic effect. In chart 2 the course of an experiment is graphically illustrated.

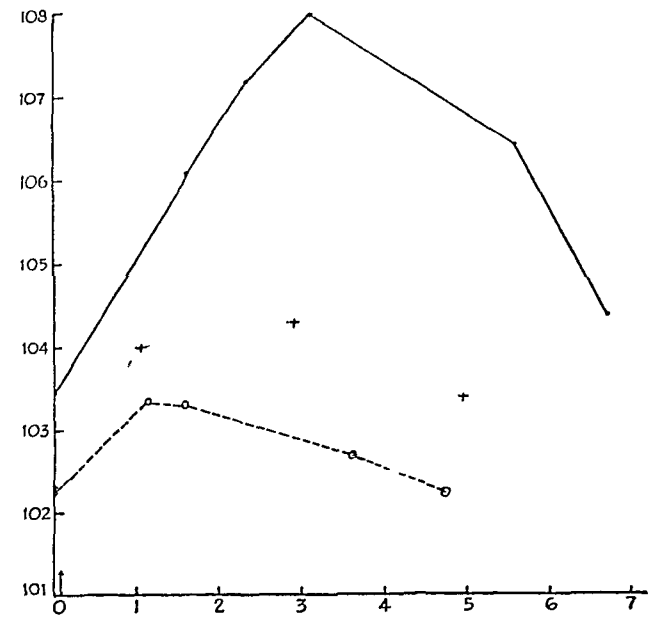


Chart 2—Effect of exudate (---+---), necrosin (—) and pyrexin (—) on the level of temperature in the rabbit. Degrees Fahrenheit are shown at the left, lapse of time in hours, at the bottom. An arrow indicates the time of injection.

TABLE 6—Dissociation of Necrosin into a Euglobulin Component (Purified Necrosin of Exudate) and a Pyrogenic Component (Pyrexin)

Experiment No	Whole Exudate			Euglobulin (Purified Necrosin)			Pyrogenic Component (Pyrexin)		
	Basal Temperature, F	Maximal Temperature, F	Increase in Temperature, F	Basal Temperature, F	Maximal Temperature, F	Increase in Temperature, F	Basal Temperature, F	Maximal Temperature, F	Increase in Temperature, F
1	102.4	104.4	2.0	102.9	103.2	0.3	102.8	104.0	1.2
2	103.5	105.2	1.7	102.3	103.1	0.8	103.2	105.7	2.5
3	102.05	104.0	1.95	103.5	104.3	0.8	102.6	104.3	1.7
4	102.0	105.1	3.1	102.3	103.1	0.8	102.3*	104.2	1.9
5†	103.3	105.6	2.3	103.3	104.1	0.8	-	-	-
6§	102.7	104.5	1.8	104.2	104.0	0	102.7	103.6	0.9
7	102.3	104.3	2.0	102.25	103.35	1.1	-	-	-
8#	102.8	105.8	3.0	103.6	105.0	1.4	103.45	108.0	4.55
9	102.4	104.75	2.35	103.9	105.5	1.6	103.05	105.6	2.55
10	103.75	106.1	2.35	103.2	104.6	1.4	103.4	106.25	2.85
11	-	-	-	103.65	104.8	1.15	102.8	105.5	2.7
12‡	-	-	-	-	-	-	102.7	106.5	3.8
Average increase in temperature			2.25	0.94			2.46		

§ There was a poor yield of exudate, and the exudate had a methemoglobin like color.  
\* The whole undissociated necrosin or the euglobulin fraction per se induced in a rabbit a rise of 1.9 F in temperature indicating thus that the pyrogenic activity lies entirely in the insoluble fraction (pyrexin) which, as shown yielded a similar rise in temperature.  
† The whole undissociated necrosin or the euglobulin fraction likewise induced a rise of 2.3 F. This supports the view that the pyrogenic factor is not in the euglobulin fraction per se since the latter yielded only a negligible increase in temperature.  
# The amount of exudate used was 0.5 cc but the corresponding amounts of euglobulin (necrosin) and pyrexin were the equivalent of about 1 cc of the original exudate.  
‡ The pyrexin utilized was previously dried by freezing.

evidence therefore seems to indicate that pyrexin is the

22 O. Smith and G. Van S. Smith (Proc Soc Exper Biol & Med 55 285, 1944) have recently stated that the euglobulin fraction of menstrual fluid contains a toxic factor. This fraction has been described by them as being insoluble in the presence of electrolytes. I have demonstrated in their laboratory with their material the marked pyrogenic activity of the toxic factor of menstrual fluid. It is quite probable that this pyrogenic activity is due to the presence of pyrexin or a similar factor in association with the toxic factor of menstrual fluid.

The toxicity of purified necrosin is readily demonstrated in its capacity to induce in rabbits a marked edematous and necrotizing cutaneous inflammation such as was previously described with the undissociated material.<sup>1</sup> Furthermore, the purified necrosin is found to be lethal in mice. Pyrexin fails to induce any cutaneous reaction, and it is innocuous in mice.<sup>23</sup>

23 A potent fraction of pyrexin has proved at times to be lethal in rabbits. In such animals after administration of the material the temperature rose to well over 108 F. Necropsy revealed primarily damage to the liver in the form of conspicuous fatty infiltration.

*Some of the Properties of Pyrexin*—The chemical nature of pyrexin remains to be determined. Studies now in progress will, it is hoped, help in clarifying this point. Meanwhile a few noteworthy determinations concerning the properties of this active pyrogenic substance are presented.

*Heat Stability of Pyrexin*—When pyrexin is heated, even to the boiling point, and then introduced into an aural vein of a rabbit, its activity remains essentially unaltered. The observations bearing on this point are listed in table 7. The trend of one experiment is shown in chart 3. The average increase in temperature with

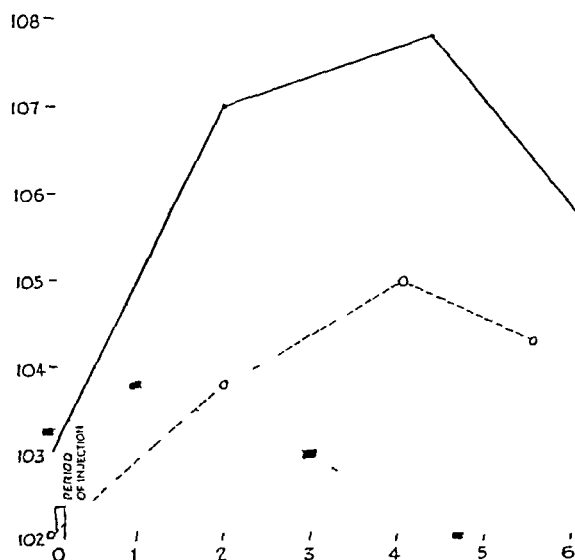


Chart 3—Heat stability of the pyrogenic factor associated with the euglobulin fraction of exudate, temperature curve with euglobulin of exudate (necrosin + pyrexin) (— — — —), curve with boiled euglobulin of exudate (————), curve with ashed euglobulin of exudate (.....). Degrees Fahrenheit are shown at the left, lapse of time in hours, at the bottom.

the boiled material was 2.34 F. Such a rise is practically of the same order of magnitude as the rise obtained with the unheated material (compare tables 7 and 3). The whole euglobulin fraction of exudate, which, as seen in the foregoing pages, contains both necrosin and pyrexin, was used in the experiments. Nevertheless, when boiled pyrexin is utilized, the pyrogenic activity is likewise maintained.

The material is inactivated when ashed, indicating that the biologic effect is not referable to an inorganic ion in the ashed residue (table 7).

Pyrexin appears to be insoluble in ether, in 95 per cent alcohol and in concentrated sulfuric acid, and, as pointed out previously, it is insoluble in distilled water or in water containing electrolytes such as sodium chloride or ammonium sulfate. It is apparently soluble in relatively weak sodium hydroxide, and it dissolves, though slowly and with difficulty, in a stronger solution of this reagent.

When purified necrosin is incubated at about 37 C. for several hours, it often yields a fraction which is actively pyrogenic. This effect is well illustrated in chart 4. Thus an otherwise relatively inactive necrosin after incubation yielded a potent fever-inducing fraction.

As pointed out,<sup>21</sup> observations on the liquefaction of a clot by necrosin indicate that the latter contains a proteolytic factor if it is not per se a proteolytic enzyme. The observations on the effect of incubation on purified necrosin add further support to the surmise that pyrexin

may well be a split product of proteolysis exerted by necrosin on a euglobulin substrate. Pyrexin fails to display any proteolytic activity on a clot induced by fibrinogen and thrombin. Its stability when heated suggests, in part at least, that it is probably not an ordinary proteolytic enzyme, in contrast with necrosin.

TABLE 7—Effect of Heat on the Activity of the Pyrogenic Factor of Necrosin

Treatment of Necrosin	Rabbit No	Basal Temperature, F	Maximal Temperature During a Period of About 6 to 7 Hr, F	Increase in Temperature, F
Untreated	23 27	103.6	105.25	1.65
	23 08*	103.0	104.4	1.4
	23 17*	102.5	107.0	4.5
	23 20	102.4	106.6	4.2
	23 11	103.0	106.8	3.8
	23 52	102.05	105.0	2.95
	23 52	103.8	105.6	1.8
	23 52	102.5	106.4	3.9
	23 52	102.6	104.5	1.9
	23 52	102.4	106.3	3.9
	Average increase in temperature			3.0
Boiled	23 17*	102.8	105.2	2.4
	23 48*	103.2	104.5	1.3
	23 26	103.2	103.65	0.45
	23 27	102.6	105.5	2.9
	23 38	103.0	107.8	4.8
	23 38	102.6	105.8	2.2
	23 38	102.7	105.6	2.9
	23 38	102.4	105.45	3.05
	23 38	102.45	103.6	1.15
	23 11	103.2	106.3	3.1
	23 52	102.65	104.1	1.45
	Average increase in temperature			2.34
Ashed	23 78	103.0	103.3	0.3
	23 72	103.6	104.7	1.1
	23 70	102.2	103.4	1.2
	23 61	102.5	102.6	0.1
	23 27	102.0	102.4	0.4
	23 72	103.25	103.8	0.55
	Average increase in temperature			0.61

\* Human necrosin was utilized, canine material was employed in the other experiments.

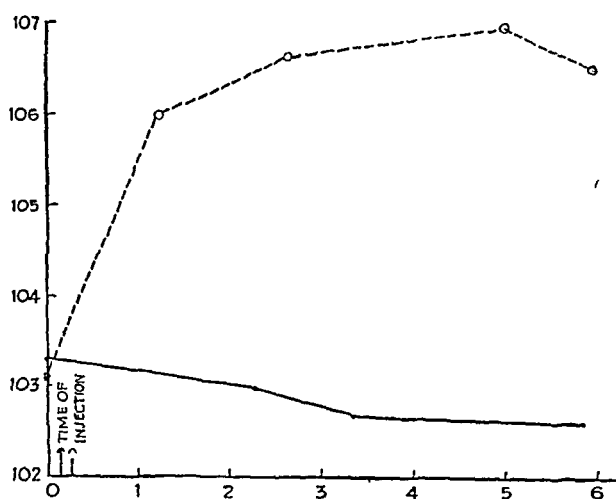


Chart 4—Effect of incubation on an otherwise non-pyrogenic purified necrosin fraction: temperature curve with nonincubated necrosin (————), curve with incubated necrosin (— — — —).

When either the whole euglobulin fraction of exudate or pyrexin is treated with crystallized trypsin and incubated, the pyrogenic effect remains essentially unaltered. Crystallized trypsin per se is nonpyrogenic. Scrapings of the duodenal mucosa of dogs or rabbits

suspended in glycerin fail to yield enzymes which appreciably affect the potency of pyrexin

Nitrogen determinations conducted by Miss Constance Sellman, of the New England Deaconess Hospital, in Boston, have yielded in three samples of pyrexin concentrations of 12.93, 10.65 and 10.23 per cent, respectively. Three total phosphorus determinations have shown concentrations of 0.79, 0.915 and 1.39 per cent, respectively. The ratio of the nitrogen to the phosphorus does not seem to indicate that one is dealing with a simple nucleic acid derivative, while at the same time the inability of trypsin to hydrolyze the active material does not seem to support a simple intermediary nitrogenous compound, e. g., a peptide, although in this respect it is to be recalled that trypsin itself has some degree of substrate specificity. The possibility is not precluded that one may be dealing with a peptide attached to a derivative of nucleic acid. Preliminary deamination may yield further information on this point. A phospholipid linkage is also not yet precluded by the available data. The Molisch test on the material is negative, suggesting the absence of any conspicuous carbohydrate grouping. The material is usually found to react positively in the ninhydrin test and to yield only a minute trace of a reaction in a biuret test. That one may be dealing with a peptide linked to a derivative of nucleic acid is a distinct possibility. This view, however, will have to be studied further before a final conclusion can be reached.

*Excretion of a Pyrogenic Factor in Urine with the Progress of an Acute Pleural Inflammation*—It is of interest to determine the fate of the pyrogenic factor with the progress of inflammation. It is found that

in part excreted in the urine (table 8 and chart 5). The active material in the urine can be frequently recovered as a precipitate by treating the urine for a number of hours in a refrigerator with ammonium sulfate at one-third or one-half saturation (chart 5). The nitrogen content of one such active sample was found to be 10.63 per cent, while the total phosphorus

TABLE 8—*Urinary Excretion of a Pyrogenic Factor During the Progress of an Acute Pleural Inflammation*

Dog from Which Urine Was Obtained	Rabbit No	Duration of Inflammation in Dog, Days	Maximal Rise in Temperature in Rabbit Within a Few Hours After the Injection of Canine Urine, F
10 F	23 26	0	1.0
	23 26	1	1.65
	23 26	2	2.8
	23 26	3	3.0
	23 26	4	2.7
11 F	23 18	0	0.5
	23 54	1	1.9
	1	1 day after reinjection of irritant in the pleura	1.75
	23 26	2 days after reinjection of irritant	2.5
12 F	23 54	0	2.35
	23 27	1	2.85
	23 27	4	3.75
14 F	X	0	2.8
	23 27	1	2.75
	23 11	2	4.1
	23 30	3	4.6

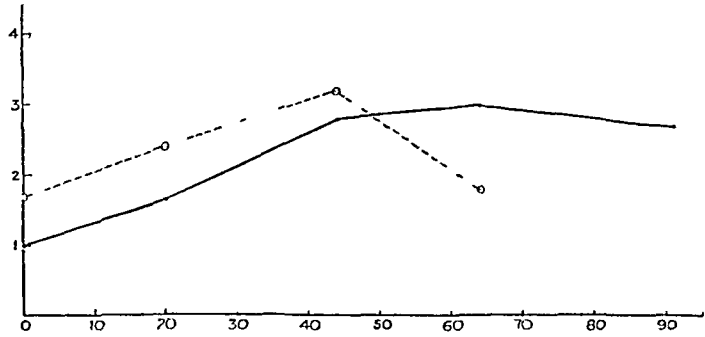


Chart 5—The urinary elimination of a pyrogenic factor in an animal with inflammation ———, temperature curve showing the presence of a pyrogenic factor in the urine, — — — —, curve showing the presence of a pyrogenic factor in the ammonium sulfate-treated fraction of the urine. Degrees of maximum increase in temperature (F) are shown at the left, duration of inflammation in hours, at the bottom.

in general the urine becomes gradually more pyrogenic as the inflammatory reaction proceeds. The data bearing on several of the experiments from which this observation is drawn are listed in table 8. The dog is kept in a metabolic cage before and after intrapleural introduction of the irritant. The urine is collected daily and about 0.5 cc is injected intravenously into a rabbit. It is to be noted that in 2 dogs (12F and 14F) the urine per se prior to inducing pleurisy in the animals is distinctly pyrogenic. This fever-inducing capacity, however, is further enhanced with the progress of the inflammatory reaction. The initial pyrogenic activity sometimes encountered with the urine of an apparently normal dog raises a question as to the possibility that pyrexin is a product of protein catabolism which under apparently normal circumstances is eliminated in the urine. This point deserves further study. At any rate, the observations suggest that pyrexin formed at the site of inflammation is at least

content was approximately 0.99 per cent. These chemical determinations on material from urine are of the same order as those found for pyrexin recovered from exudate. This perhaps suggests that one is dealing with a single substance formed at the site of injury and subsequently excreted at least in part in the urine.

*Mode of Action of Pyrexin*—The exact mode of action of pyrexin doubtless requires considerable further study. Preliminary observations indicate that an antipyretic such as acetylsalicylic acid apparently reduces the activity of pyrexin, as demonstrated when it is subsequently injected into a rabbit. Antipyretics are believed to act directly on the centers regulating temperature. Furthermore, it is known that barbiturates depress the hypothalamic region near where the temperature-regulating centers are presumably located.<sup>24</sup>

24 Masserman, J. H. Arch. Neurol. & Psychiat. 37: 617, 1937.



Pentobarbital sodium administered to a rabbit that has received the whole euglobulin fraction of exudate or pyrexin tends to depress the pyrogenic activity (chart 6). These facts are merely suggestive that the effect of pyrexin is directly on the fever-controlling centers located in the region of the hypothalamus.

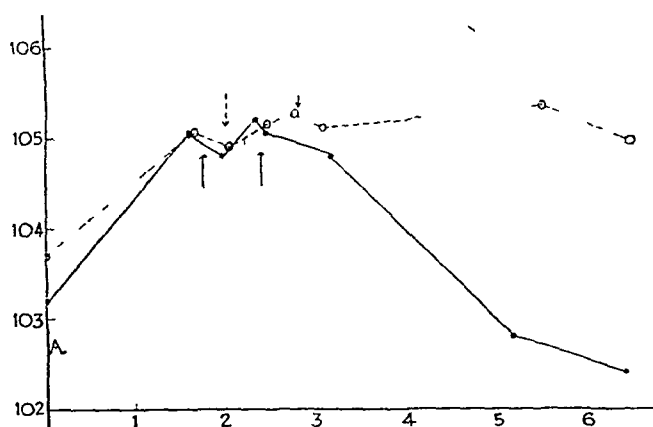


Chart 6—Depressing effect of pentobarbital sodium on the pyrogenic action of pyrexin: temperature curve when pyrexin and isotonic solution of sodium chloride were injected (— — — —), curve when pyrexin and pentobarbital sodium in isotonic solution of sodium chloride were injected (————). Degrees Fahrenheit are shown at the left, lapse of time in hours, at the bottom. The arrows indicate the points at which injections of pyrexin (— — — —>) and saline solution (— — — —>) and pentobarbital sodium in saline solution (————>) were made.

#### COMMENT

The observations reported in the present communication indicate that there is a pyrogenic factor in inflammatory exudate. This factor is associated with the euglobulin fraction but evidently is not itself a euglobulin. It seems rather to be a proteolytic split product brought about by the action of necrosin, which in turn either is an enzyme or in its present state of purification displays proteolytic activity. Purified necrosin as such is nonpyrogenic. It seems to be either a true euglobulin or at least a substance linked with that protein fraction of exudate. The fever-inducing substance is found to be largely associated with the relatively insoluble component of the whole euglobulin fraction of exudative material. With respect to this insolubility in the presence of  $\text{SO}_4^{=}$  ions it is in contrast with necrosin. The latter is soluble also in isotonic solution of sodium chloride and appears to form at least part of the euglobulin complex of exudate. For the sake of convenience, the pyrogenic factor has been termed pyrexin.

The exact chemical nature of pyrexin is still unknown. It seems to be an end product of enzymatic activity associated with necrosin. Its nitrogen concentration is in the neighborhood of 11 per cent and its total phosphorus concentration is about 1 per cent. The material is insoluble in distilled water, in isotonic solution of

sodium chloride and in the presence of ammonium sulfate. It is insoluble in a strong acid but appears to be soluble in a relatively weak alkali. It is indiffusible, and it is heat stable. The ninhydrin reaction on pyrexin is frequently positive, although this does not appear to be true of the active material recovered from urine. The Molisch test for carbohydrates is negative. The possibility that one is dealing with a simple peptide attached to a nucleic acid group is not as yet precluded by the data on hand. Further studies are under way in an endeavor to obtain more precise information concerning the chemical nature of pyrexin.

In earlier studies I pointed out that the basic phenomena in inflammation are referable to the liberation of various biochemical units.<sup>1b</sup> These common factors result probably from a derangement or an alteration of the chemical organization of the cell imparted or initiated by the presence of an irritant. It is these factors which are responsible for the essentially stereotyped reaction of inflammation. Such liberated substances include leukotaxine, capable of reasonably explaining the increased capillary permeability and the local migration to the site of injury of polymorphonuclear leukocytes<sup>25</sup>, the leukocytosis-promoting factor, which explains the leukocytosis often associated with inflammatory processes and the concomitant hyperplasia of granulocytes in the bone marrow,<sup>26</sup> and necrosin (possibly a proteolytic enzyme), which is capable of accounting for the primary mechanism of injury in the development of inflammation.<sup>1</sup> Dextrose and possibly even urea are likewise liberated by injured cells into exudative material.<sup>27</sup> Finally, a growth-promoting factor or factors perhaps concerned with repair seem to be released into the exudate.<sup>28</sup>

Fever, as a rule, is associated with some form of cell injury. The present study indicates the presence in an inflamed area of pyrexin, which is concerned in the production of fever with inflammation. Its mode of action may be a direct effect on the heat-regulating centers in the hypothalamic region. In brief, all these studies point to the necessity of improving further the present understanding of the biochemical nature of the injured cell in order to throw additional light on a number of basic pathologic processes.

25 Menkin, V. *J. Exper. Med.* **67** 129 and 145, 1938, *Dynamics of Inflammation*, New York, The Macmillan Company, 1940.

26 Menkin, V. *Am. J. Path.* **16** 13, 1940, *Arch. Path.* **30** 363, 1940, in Alexander, J. *Colloid Chemistry*, New York, Reinhold Publishing Corporation, 1944, vol. 5, *Am. J. Path.* **19** 1021, 1943.

27 Menkin, V. *Am. J. Physiol.* **138** 396, 1943.

28 Menkin, V. *Cancer Research* **1** 548, 1941.



## SUMMARY

A pyrogenic factor is associated with the euglobulin fraction of inflammatory exudate. To a large extent it can be dissociated from necrosin, a toxic material, which in turn either is a euglobulin or is at least a substance closely linked with that protein fraction of exudate.

The dissociation of the pyrogenic factor from necrosin is accomplished by differential solubility. Necrosin is soluble in water containing sulfate ions, whereas the pyrogenic factor fails to be appreciably dissolved in the presence of such ions.

The pyrogenic factor is termed, for the sake of convenience, *pyrexin*. Its exact chemical nature is still problematic. It appears to be a hydrolytic product brought about by the action either of necrosin or of proteolytic enzymes associated with the latter.

Pyrexin is nondiffusible, and it is heat stable. Its nitrogen and phosphorus concentrations are about 11 and 1 per cent, respectively.

Pyrexin is essentially absent in normal non-hemolyzed blood serum, but it is present to some extent in hemolyzed serum and also in the serum of an animal with a concomitant inflammation.

In experiments on dogs a pyrogenic factor was recovered in increasing amounts from urine with progress of an inflammatory reaction in the pleural cavity. The evidence suggests that one is dealing with pyrexin, which is eliminated at least in part in the urine.

Pyrexin offers a reasonable explanation for the primary mechanism of the fever frequently accompanying inflammation. Preliminary indirect studies suggest that its mode of action may possibly be via the hypothalamic heat-regulating centers.

# CLASSIFICATION OF TUMORS OF THE KIDNEY

GEORGE L. FITE, M.D.

BETHESDA, MD

This is a study of over 600 renal tumors, 50 of which are from the Pathology Laboratory of the National Institute of Health, the remainder are in the Kidney Tumor Registry at the Army Medical Museum. Of these tumors, five hundred and eighty-six may be roughly classified as follows:

Tumors derived from pelvic epithelium	114
Mixed (Wilms) tumors, adenosarcoma, etc	61
Miscellaneous tumors	15
Papillary and solid cancerous tumors	396

## TYPES OF PAPILLARY AND SOLID RENAL CANCERS

This article deals particularly with the 396 tumors of the last category, which embraces all the more disputable renal tumors. Studies for fat and glycogen were made on suitable sections of 75 of these. Somewhat less than half the total (191) can be readily classified as of four recurring types:

Group I 29 tumors bearing a close resemblance to the common noncancerous adenoma of the renal cortex.

Group II 85 papillary carcinomas containing both glycogen and lipoids, with clear cells often predominant.

Group III 13 solid carcinomas composed of large, finely granular cells with glycogen but not lipid.

Group IV 64 solid carcinomas with cells grouped in small cords or islands.

The remaining 205 tumors are chiefly mixtures of the types mentioned in which variations make precise classification difficult.

*Group I*—The nomenclatures which have been employed by pathologists for the cancerous tumors derived from the cortical adenomas are particularly confusing. The cancerous tumors have been called papillary or tubular carcinoma, or adenocarcinoma, or they have been called granular cell, solid cell or smooth cell carcinoma, to distinguish them from clear cell tumors. Unfortunately, these same terms are often applied to other types of cancer not allied to cortical adenoma.

The similarities between the cancerous and the noncancerous (adenomatous) tumor of the renal cortex are sufficiently great that a description of one is almost a description of the other.

It is only needed here to emphasize that in both cancerous and noncancerous tumors the cell margins are not distinct, the inner (lumen side) margin of the papilla (or septum, according to Sudeck<sup>1</sup>) is even and regular, and the main growth takes place in the cystadenomatous manner.

Because noncancerous adenomas arise so often in scars and in arteriosclerotic contracted kidneys, multicentric origin of cancerous tumors of this type is likewise possible. In the present series there are no specimens which illustrate this clearly.

*Group II*—The archetype of tumors categorized here is the well known papillary clear cell carcinoma, the most common of all renal tumors. Many tumors of this type show papillary forms quite like those of the tumor in group I except for three important points:

1 The inner (lumen side) margin of the papilla (septum, tubular and acinous formation) is uneven and irregular.

2 The margins between cells are sharply outlined.

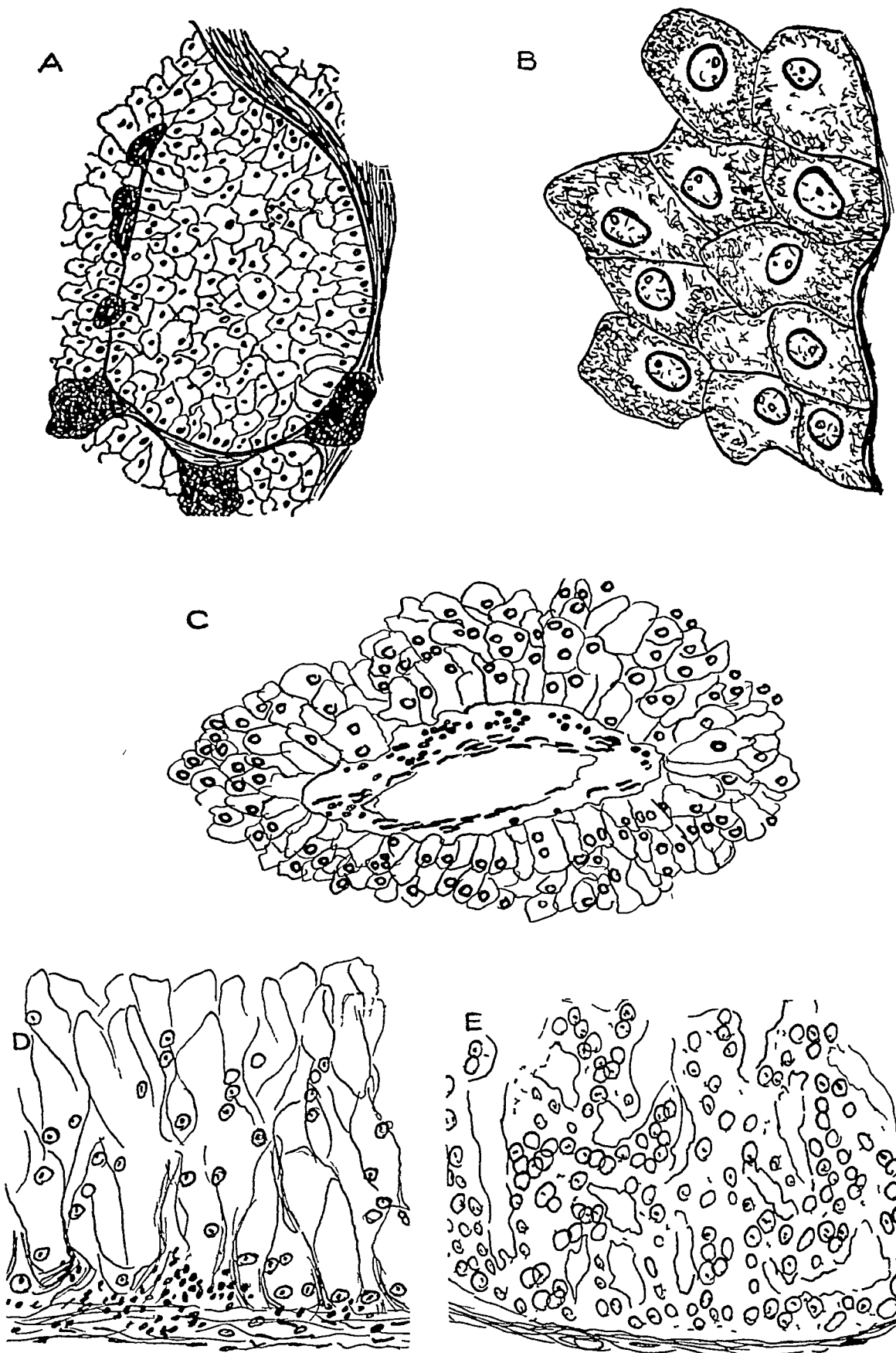
3 The cytoplasm contains abundant quantities of glycogen and of neutral and anisotropic lipid.

*Group III*—The tumors in this group represent an entity which has apparently not been recognized as such in the past and requires a brief description. It is illustrated by Ewing<sup>2</sup> under the title of "adrenal tumor of kidney." It is a solid tumor, the cells of which are finely granular and never clear except for clear halos about the nuclei (*A* and *B* in the accompanying figure). The remainder of the cytoplasm shows numerous ill defined threads and filaments, which appear as granules when cut across. Areas of tumor are seen in which these filaments seem to float in a watery cytoplasm. This cytoplasmic character is only superficially like that of the adrenal cortex, in which strands of cytoplasm separate numerous small fat droplets. Glycogen is uniformly present in all neoplastic

<sup>1</sup> Sudeck, P. Virchows Arch f path Anat **133**, 405, 1893.

<sup>2</sup> Ewing, J. Neoplastic Diseases, ed 4, Philadelphia, W. B. Saunders Company, 1940, p 820.

From the Pathology Laboratory, National Institute of Health, United States Public Health Service.



A and B, group III carcinoma of the kidney C, D and E haphazard, pseudocolumnar and syncytial multi-layered tumor epithelium lining papillae

cells as evenly scattered small granules in considerable numbers. Fat was wholly absent from the tumor cells of 4 of the tumors in this group, in a fifth a moderate amount was present in not too well nourished areas. However, much neutral fat in fine granules is widely and evenly distributed throughout the stroma of the typical tumors, the stroma being composed of coarse dense fibrous tissue in which run markedly hyaline blood vessels.

*Group IV*—This group of renal cancers is comprised mainly of tumors of the small cell solid type, commonly called hypernephroma, which shows much glycogen and lipid in the tumor cells and has a delicate stroma.

#### PAPILLARY AND SOLID RENAL CANCERS NOT READILY CLASSIFIABLE

The difficulties that are encountered in classifying the remaining 205 tumors stem from the numerous variable features which play a part in their formation. These are detailed briefly.

*Formation of Solid Areas in Papillary Tumors*—The most common variations in renal tumors are combinations of papillary structures (group II) with solid tumor tissue typical of the tumors in group IV. Two important features are associated with this process of solidification—the irregularity of the papillary margins, and the formation of multiple layers of tumor cells lining the papillae. All stages and degrees of transition and intermingling have been encountered in this series. The size of the solid "islands" formed in the papillary tumors appears to be a factor of the thickness of cell layers lining the papillae.

*Multilayered Tumor Epithelium Lining Papillae*—The cells lining these papillae take any of three common arrangements: (1) haphazard—piled on top of each other (*C* in the figure), (2) pseudocolumnar (*D* in the figure) and (3) syncytial (*E* in the figure). It has not been found practical to make subdivisions according to these cell arrangements. The cancers with the syncytial arrangement are most interesting and resemble in some ways the uncommon papillary cancers arising in the renal pelvis. Thirteen examples are noted here.

*Primary and Secondary Papillae*—Four tumors in group I and 8 in group II showed abundant short secondary papillae lining the longer primary papillae. In the tumors of group II these secondary papillae commonly anastomose.

*Granularity of Cells*—Granular cells are present in most tumors of groups II and IV. Clarity of cells is chiefly the effect of accumulation of

fat and glycogen. However, cells may contain both these substances in fair quantities in the form of small granules and still exhibit granular cytoplasm. The clarity of the cells is of no moment, it is a matter of the cell contents. Hence classification on the basis of granularity or clarity of cells is not workable.

*Glycogen*—Tumors of group I and tumor tissue of group I type intermingled with other types do not reveal glycogen. It occurs in tumors of groups II and IV usually as large and small globules or masses, sometimes as pseudomaltese crosses. In tumors of group III it is granular in form, exclusively.

*Lipoids*—Cholesterol clefts occurring in necrotic and inflammatory areas, occasionally with some xanthomatoid reaction, are of little or no significance. Neutral fat deposits are common in poorly nourished areas. The best guide to the importance of fat deposits is the proportion of doubly refractile lipid present. In some tumors with relatively little fat along the basal margin of the cells, the proportion of anisotropic material is high. Significant fat deposits are accompanied by extensive glycogen deposits. The apparent sequence is—glycogen → neutral fat → anisotropic lipoids. The studies of Wells<sup>3</sup> and other authors have shown most of the doubly refractile lipid to be cholesterol and lecithin.

*Xanthoma Cells*—The stroma of 26 tumors showed such cells.

	Cases	Percentage
Wilms tumors	2	4
Noncancerous cortical adenomas	12	37
Group I, cancerous	8	28
Group II	4	5

The percentages are based on the totals of the readily classifiable tumors. Xanthoma cells were not seen in any others. Schiller<sup>4</sup> considered these cells metaplastic adrenal cells, but Ribbert<sup>5</sup> and others have found them especially in cortical adenoma. The xanthoma cells do not appear to be neoplastic in character.

*Desmoplasia*—This was seen in 18 solid tumors of group IV, usually in isolated areas, sometimes involving much of the tumor. Inter-cellular fibrosis is about equally common in the more static forms of solid tumors of group IV noninvasive in appearance. It distorts the arrangements of cells in cords and islands to a marked degree.

3 Wells, H. G. J. M. Research **17** 461, 1907-1908

4 Schiller, W. Arch. Path. **33** 879, 1942

5 Ribbert, H. Geschwulstlehre, ed. 2, Bonn, F. Cohen, 1914, p. 547

*Anaplasia*—This may occur in any type of tumor tissue. In 18 tumors it was so extensive that analysis of their basic character was impossible. Isolated anaplastic areas are common in many tumors and are the most frequent source of metastases.

*Capsules*—Though often observed in renal tumors, capsules are without significance. Tumors of groups II and III are never infiltrative unless anaplastic. This is probably also largely true of those of groups I and IV. The tendency to grow in a cystadenomatous manner is pronounced in the case of papillary growth and is not lost in the solid tumors. No conclusions should ever be drawn as to the liability of these tumors to spread by metastasis on the basis of the presence or the absence of a capsule.

*Lumens*—Wherever the solidification of a papillary tumor is imperfect, lumens may be expected. They will not have regular outlines, because, as indicated in an earlier paragraph, the lumen side margins of the tumors of group II are characteristically irregular. Potential lumens which exist in solid tumors are often made apparent only by hemorrhage in them or extravasation of fluid. Thirteen tumors of groups II and IV were predominantly cystic as a result of this.

*Chronic Inflammatory Changes*—Extensive lymphocytic and plasma cell infiltrations were observed in 11 tumors of group II with multi-layered epithelium lining the papillae. The inflammatory changes were not related to necrosis or degeneration.

*Wilms Tumors Occurring in Adults*—These may show the papillary type of renal cancer. Three excellent examples were noted as follows: (1) papillary clear cell cancer intermingled with rhabdomyosarcoma, (2) group I, II and IV tumor plus myoblastic sarcoma and (3) cystic group II cancer together with large quantities of adult smooth muscle.

*Embryonal Carcinoma*—In addition to the mixed (Wilms) tumors, there are other embryonal tumors which also appear to have arisen from the renal blastema, composed of darkly staining cells. Seven solid and 2 alveolar cancers of this type occurred in the series. The term "embryonal carcinoma" should be limited to these rare tumors.

*Adenocarcinoma*—In the recapitulation to follow 15 tumors diagnosed as adenocarcinoma are listed under group I. Adenocarcinoma of the kidney does not differ greatly from that of the stomach, the breast and other organs. Nine of these tumors show some atypical traces of group I type tumor tissue. Six cannot be iden-

tified as renal tumor on a histologic basis. The use of the term "adenocarcinoma" should be avoided elsewhere in the nomenclature of renal tumors.

*Opaque Cell Papillary Carcinoma*—There are 7 tumors in the series in which the character of the cells is sufficiently different to set them apart as a subgroup of group I. They are smooth cell, opaque cell or solid cell cancers. The cells are larger from a broadening out of the cytoplasm, which is basophilic and pale but without any trace of granulation. The nuclei have prominent nucleoli, and in 1 tumor there are numerous hyaline eosinophilic cytoplasmic bodies.

*Age and Sex Incidence*—Only the more readily classifiable tumors on which data are available are listed.

	Percentage			
	From Males	From Females	from Males	Average Age
Group I	38	5	88	51.8
Group II	120	35	77	54.4
Groups III and IV	67	40	63	54.1

Owing to the fact that the male population was somewhat more heavily drawn from than the female, some correction would have to be made, but this still would leave a preponderant incidence of tumors of groups I and II in males. The ages of occurrence range widely from 40 onward.

*Recapitulation*—Although the variations in individual tumors often render classification awkward, there exists a pronounced tendency, even on the part of some highly differentiated solid tumors, to hark back to the noncancerous papillary adenoma. Overlapping of features with those exhibited by cancerous tumors of both mesonephroic and pelvic origin leads necessarily to the conclusion that cells of adrenal gland origin play no part whatever in their histogenesis.

The classification of the 627 tumors of the kidney may be recapitulated as follows:

A	Tumors derived from pelvic epithelium	114
B	Tumors derived from the renal blastema	
	1 Mixed tumors—adenocarcinoma, adenocarcinoma, Wilms tumor	51
	2 Apparently pure myosarcoma and myoblastoma	10
	3 Embryonal carcinoma	9
C	Miscellaneous tumors—fibrosarcoma, symphaticoblastoma, hemangiosarcoma, etc	15
D	Tumors derived from the renal glandular epithelium	
	Group I Adenopapilloma and its cancerous derivatives	

A Noncancerous and adenopapilloma	32
B Papillary and tubular carcinoma	29
C Opaque cell papillary carcinoma	7
D Adenocarcinoma	15
Group II Papillary and partly solidified carcinoma containing glycogen and fat	
A Wholly papillary carcinoma	85
B Papillary and solidified carcinoma	90
C With added features of group I carcinoma	25
Group III Large, finely granular cell carcinoma containing glycogen in granular form	
A Uniformly solid	13
B With some papillary forms	11
C With added features of group I carcinoma	3
Group IV Solid tumors	
A Uniformly solid, small cell groups	64
B Uniformly solid, large cell groups	13
C Anaplastic	18
D With added features of group I carcinoma	10
Group V Miscellaneous	
A Mixed tumors showing group II papillary carcinoma (Wilms tumor in adults)	3
B Tumors dubiously of pelvic or renal parenchymal origin	3
C Noncancerous adenoma of duct of Bellini	1
D Tumors unclassifiable because of extreme variation	6

## SUMMARY

The tumors derived from the adult renal parenchyma have the common feature of a basic papillary structure. Although the uniformly solid tumors are wholly solid from their beginnings, they nonetheless repeat structural forms observed in the papillary tumors which have become solid in some parts. Thus, in the transitions from the purely papillary to the purely solid tumors there seems to be a development or evolution as a result of which any combination of different types of tumor cells or tissues may occur. This evolutionary phenomenon is remarkable for three important features, viz, the accumulations of glycogen and lipoids, the alterations in character of the cytoplasm of the tumor cells and the filling-in of lumens or inter-papillary spaces to form islands of solid tumor tissue.

In spite of the numerous variable factors in the papillary tumors, even the more rare features are observed repeatedly in an adequately large series, and, in addition, it is found that about half of these tumors can be readily placed in one or another of four groups representing four distinct cellular and structural types.

# Case Reports

## FATAL PANNICULITIS

CAPTAIN NATHAN B. FRIEDMAN

MEDICAL CORPS, ARMY OF THE UNITED STATES

Although a number of cases of Weber-Christian disease have been recorded,<sup>1</sup> reports which include postmortem observations are rare.<sup>2</sup> It is the purpose of this paper to present the clinical and the pathologic observations in another case of fatal panniculitis.

### REPORT OF A CASE

A 23 year old white salesgirl from the San Joaquin Valley of California was first seen at the Stanford University Hospitals in 1938. She complained of recurrent crops of painful red spots and elevated nodules on her legs. Their first appearance was associated with fever, generalized aching and cough. During 1937 she had several crops, accompanied by elevation of temperature to 103 F, night sweats and fatigue. During one episode, in August, she also had abdominal pain and vomiting. In December she required hospitalization for cellulitis of the jaw following extraction of a tooth because of "focal infection."

In April 1938, at another institution, one of the lesions cleared up under treatment with local injections of thymol and its administration by mouth, this therapy was instituted in the belief that the disease was an infection with *Coccidioides*. However, another nodule on the leg broke down, and fresh lesions appeared despite treatment.

**Examination**—The heart and the lungs appeared normal. The blood pressure ranged from 90 to 114 mm of mercury systolic and from 50 to 70 diastolic. The spleen was palpable 2 fingerbreadths below the costal margin, but the abdominal findings were otherwise not remarkable. The ankles appeared swollen, but there was no pitting edema.

There were many lesions ranging up to 3 cm in diameter on the lower parts of the legs, especially on the lateral and posterior aspects of the calves. There were a few on the thighs and buttocks and one in the left breast. Some were dark purple-red blotches, while others were indurated nodules fixed to the skin but not to the deep tissues. In places the overlying skin was scaling. A few lesions on the legs showed ulceration with formation of superficial sinuses and a purulent discharge. The sites of some receded nodules appeared as depressed scars.

**Course**—Because of low titers of antistaphylolysin and antihemolysin the patient was given a graded

series of injections of staphylococcus toxoid, and the titers rose to within the normal range. At first the injections were followed by chills and fever, with swelling of the joints of the fingers. New nodules appeared and some broke down, but eventually the ulcers healed.

After a remission which lasted until March 1939, fresh lesions appeared. In June the patient removed a crusted "keratosis" near her left ankle with a razor. It was not clear whether this area had been the site of one of the nodules. However, an ulcer, resistant to local treatment, developed, it persisted for months and was associated with bouts of fever. It became much worse in July 1940, after cauterization at another institution. In November 1940, while she was in Stanford Hospital for treatment of the lesions on the legs, staphylococcal septicemia developed. Sulfathiazole had to be administered for six days before blood cultures became sterile. There were splenomegaly and leukopenia. By January 1941 the ulcer on the ankle had healed and the spleen had become smaller.

During most of 1941 the patient did fairly well and her leukocyte count was as high as 6,000 cells per cubic millimeter of blood. However, by the end of the year rehospitalization was made necessary by the appearance of fever, subcutaneous nodules and a painful swelling in the left breast. The temperature was as high as 102.2 F, and the spleen again extended 4 fingerbreadths below the costal margin.

She was hospitalized for the last time Feb. 26, 1942, with a temperature of 105.8 F and a leukocyte count of only 1,000 cells per cubic millimeter of blood. A lesion on the inner upper portion of the left thigh and buttock had ulcerated and enlarged until it measured 10 by 15 cm. The surrounding induration extended up over the inguinal region. The lower portion of the abdomen became exceedingly tender, and the temperature ranged between 104 and 105.8 F. She did not tolerate food and was kept alive by intravenous injections of fluids and transfusions. A systolic murmur and a gallop rhythm appeared, the heart sounds grew faint and the cardiac shadow was enlarged on roentgen examination. Between March 10 and March 20 she was given 6 roentgen baths of 5 r each. She died, disoriented and semicomatose, March 25, with a terminal temperature of 106.7 F.

**Laboratory Data**—Urinalysis repeatedly gave normal results, and the Wassermann reaction of the blood was negative. The hemoglobin content of the blood ranged from 111 to 149 Gm per hundred cubic centimeters. Erythrocyte counts varied between 3,700,000 and 5,100,000 cells per cubic millimeter of blood. Examination of marrow, obtained by sternal puncture, revealed moderate cellularity, there was slight reduction of the myeloid elements but normal distribution. The red cell series was normal. Many tuberculin tests made with dilutions of 1:10,000 and 1:1,000 gave no reactions. There were repeated, strongly positive reactions to coccidioidin (0.1 cc of a 1:1,000 dilution), the site

From the Departments of Pathology and Medicine, Stanford University School of Medicine.

1 Larkin, V. de P., DeSanctis, A. G., and Margulis, A. E. *Am J Dis Child* **67** 120, 1944.

2 (a) Tilden, I. L., Gotshalk, H. C., and Avakian, E. V. *Arch Dermat & Syph* **41** 681, 1940. (b) Miller, J. L., and Kritzer, R. A. *ibid* **47** 82, 1943. (c) Spain, D. M., and Foley, J. M. *Am J Path* **20** 783, 1944.

of injection was marked by an area of erythema 4 cm in diameter with an indurated center 2 cm in diameter. Material from the lesions on the legs was cultured on Sabouraud's medium and other mediums and was inoculated into guinea pigs (intraperitoneally and intratesticularly). *Coccidioides* in particular was searched for. No mycotic or acid-fast forms were found, although both *Staphylococcus albus* and *Staphylococcus aureus* were encountered, some of which were coagulase positive. A portion of material from the nodule in the breast was also cultured and inoculated into guinea pigs, with negative results.

The following agglutination tests gave negative results: typhoid bacilli (H and O), paratyphoid bacilli, *Bacillus supestrifer*, *Brucella abortus*, *Brucella suis*, *Brucella melitensis*, *Bacillus tularensis* and *Bacillus proteus* X 19. The reaction to brucellergin in a cutaneous test with 0.1 cc of antigen was negative. Cultures of stools yielded no organisms of the typhoid-dysentery group. Chemical examinations of the blood gave the following values: protein, 5.2 to 5.7 Gm, calcium, 8.3 mg, phosphorus, 3.9 mg, sugar, 82 to 93 mg, urea, 18 to 21 mg, and cholesterol 188 mg per hundred cubic centimeters. The phosphatase content of the blood was 6.7 units (Bodansky).

White blood cell counts showed leukopenia during exacerbations of the disease, despite complications such

lymphocytes or large stellate, spindle or rounded elements resembling macrophages. Mitotic figures, some of which were of the radial "burst" type, were present in the cells of the inflammatory infiltrate. Occasional large elements somewhat resembling Reed-Sternberg cells and a rare giant cell were also seen. Relatively few of the many macrophages contained fat, collections of them filled occasional lymphatics. Some epithelioid cells clustered in granuloma-like concentric rings about empty spaces which suggested that fat had been dissolved from them. Other little granulomas resembled tubercles, with central necrosis and fragmentation of chromatin. Some foci of liquefaction were present. A variety of special stains failed to reveal bacterial or mycotic organisms, including acid-fast forms.

The septums of connective tissue between the fat lobules were unmistakably less involved than the fat lobules themselves, although they did show some edema, exudation of fibrin and cellular infiltration. In the dermis the adipose tissue about the appendages was inflamed. The wall of a large vessel coursing in a fibrous septum showed cellular infiltration, edema and slight disorganization of the lamellae. There were concentric rings of proliferated adventitial cells around other vessels. These changes did not suggest primary arteritis or phlebitis, they were probably secondary to

*Representative Blood Counts During Remissions and Relapses*

Date	Condition	Leuko cytes per Cu Mm	Polymorphonuclear Leukocytes, per Cent		Lympho cytes, per Cent	Mono cytes, per Cent	Eosino phils, per Cent	Baso phils, per Cent	Blood Platelets per Cu Mm
			Immature	Adult					
July 1938	Lesions active	2,200	46	9	37	6	1	1	365,000
Oct 1938	Improvement	3,600	24	26	52	16	1	1	
Nov 1940	Septicemia	1,700	24	18	52	2	2	2	167,000
Dec 1940	Improvement	4,300	30	15	39	10		6	408,000
Nov 1941	Relapse	2,500	42	4	47	7			141,000

as the staphylococcal septicemia. The table lists some typical counts. The number of blood platelets also decreased during relapses, but the sedimentation rate was never elevated. It varied between 4 and 10 mm, with a packed cell volume of 32 to 36 per cent.

**Biopsies.**—Biopsy of an open lesion on the right leg and of a subcutaneous nodule from the left thigh was made July 25, 1938. There was chronic granulomatous inflammation predominantly involving the subcutaneous fat lobules. Round cells and large mononuclears were most abundant. The dermis was far less involved than the underlying fat. Cystic epithelial structures which represented either keratinizing epidermal inclusions or dilated follicles plugged with keratin were seen in relation to the epidermis.

A specimen obtained from a nodule in the left breast Nov 3, 1941 showed only a few large mononuclear cells and lipid phagocytes. The section studied was probably not representative since most of the lesion was used for culture and for animal inoculation experiments.

Material was taken from the edge of the large ulcer on the left thigh March 4, 1942. Sections showed a partially necrotic, ulcerated epithelial surface lined by dense connective tissue heavily infiltrated with polymorphonuclear leukocytes. Away from the ulcer the inflammatory process was most intense in the fat lobules, which showed considerable destruction of the normal adipose tissue accompanied by cellular infiltration and proliferation (fig 1). There were relatively few polymorphonuclear leukocytes, most of the cells were

the inflammation of the fat. Inflammation and thrombosis of vessels were evident in the wall of the ulcer. Spreading down from the surface epithelium and extending between the collagenous bundles of the dermis was an extensive infiltration of cords and strands of squamous epithelial cells. Many of the infiltrating cell cords appeared to be arranged along the course of a hair follicle. The cells showed mitotic figures and foci of keratinization.

**Autopsy.**—On the legs were a number of depressed, atrophic pigmented scars. The most striking feature was a foul-smelling ulcer, 15 cm in diameter, on the medial surface of the left thigh just below the gluteal fold. The surrounding tissue was indurated, swollen and purple-red. The necrotic ulcerative lesion penetrated deeply into the underlying tissues and extended upward as a fluctuant subcutaneous swelling beneath the inguinal ligament. The overlying skin was discolored, and there were several sharp-edged perforations about the main ulcer. The abdominal wall was thickened in the left lower quadrant by extension of the process. The muscle and fibrofatty tissues, which were hemorrhagic and softened, contained pockets of purulent exudate, these changes were observable through the intact parietal peritoneum. The left inguinal nodes were enlarged, firm and embedded in indurated fat, but there was no generalized adenopathy.

The pericardial sac contained 200 cc of clear fluid with a green tinge. The fluid was tested for bile, with negative results. At the apex of the left lung, beneath



a solitary fibrous adhesion, there was a fibrocalcific nodule 0.4 cm in diameter. At the base of the right lung was a subpleural hemorrhagic patch 0.5 cm in diameter. The bronchial mucosa was reddened and roughened. The liver, which weighed 2,200 Gm, contained a single ill defined yellow focus 1 cm in diameter, bulging from the cut surface. The heart

the involved portion of the abdominal wall and the spleen all yielded a coagulase-positive variety of *Staph aureus*.

*Microscopic Examination*—Thigh The epidermis was elevated from the hyperemic papillae by edema and was missing in some places. A few keratotic plugs were present, and one hair follicle was surrounded by radiat-

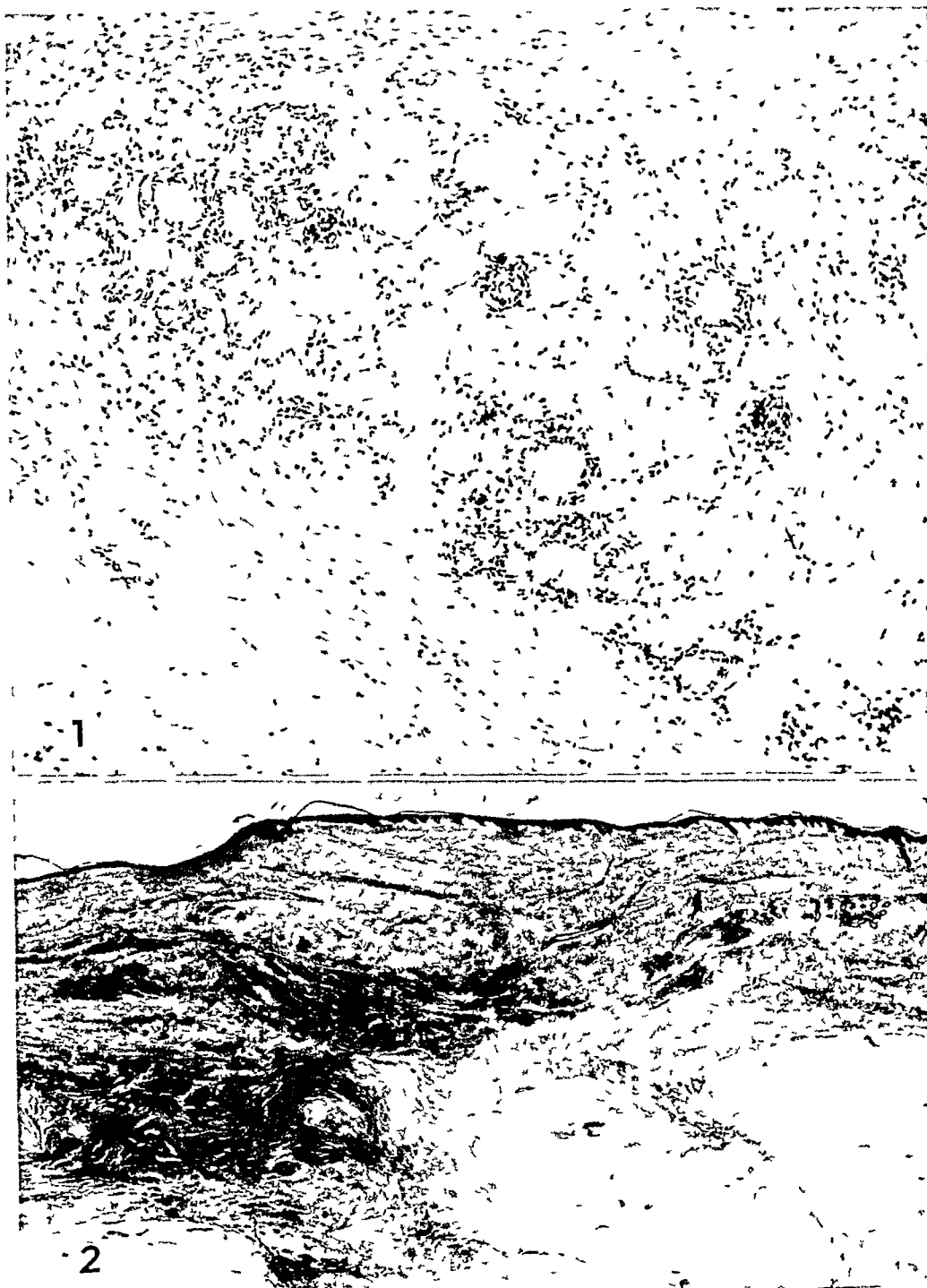


Fig 1—Inflammation of a fat lobule in a biopsy specimen removed from the thigh

Fig 2—Scar of a healed lesion from the leg

weighed 280 Gm, the spleen, 350 Gm, each kidney, about 200 Gm, and the brain, 1,400 Gm. No gross abnormalities of these organs were evident. The bone marrow, the endocrine glands and the gastrointestinal and genitourinary systems appeared normal.

*Bacteriologic Examinations*—Cultures of the blood taken from the heart post mortem and material from

ing streamers of hyperplastic epithelium comparable to those seen in one of the biopsy specimens. The ulcer was lined by necrotic hemorrhagic tissue which was infiltrated with polymorphonuclear leukocytes and contained thrombosed vessels. Away from the ulcer the lobules of adipose tissue beneath the intact epidermis were infiltrated by inflammatory cells. The fat sur-

rounding the dermal appendages was similarly involved. The cells, which were predominantly large mononuclears and macrophages, were concentrated especially about the margins of the lobules. The intervening fibrous septums were much less heavily infiltrated than the fat. The macrophages contained red cells, lymphocytes, polymorphonuclear leukocytes, chromatin debris and a few fatty vacuoles. There were occasional eosinophils, polymorphonuclear leukocytes and a few rounded hyaline acidophilic bodies.

**Abdominal Wall** The muscle and the fibrofatty tissues were infiltrated with polymorphonuclear leukocytes and mononuclears. Focal hemorrhage, necrosis and suppuration were present. Gram-positive cocci were noted. About some of the vessels there were concentric lamellas of proliferated adventitial cells and fibroblasts.

**Inguinal Node** The tissue was depleted of lymphocytes, but there was hyperplasia of the reticuloendothelial cells, many of which contained hemosiderin. The sinusoids were wide and contained many red cells, primitive lymphoid cells, large mononuclears and eosinophils. There were macrophages containing red cells, leukocytes, nuclear debris and vacuoles. A few eosinophilic bodies resembling those in the thigh were seen.

**Scar on the Leg** The section was taken from the site of an involuted nodule. The atrophic epidermis dipped down over a fibrous scar which replaced the dermis and underlying adipose tissue (fig 2).

**Lungs** There was a fibrinopurulent exudate in the bronchi, and a few patches of bronchopneumonia were noted. The hemorrhagic nodule seen grossly proved to be a milary septic infarct with a central vessel which was plugged by fibrin and contained masses of gram-positive cocci. The surrounding tissue was necrotic. The apical fibrohyaline and calcific nodule enclosed a few ovoid refractile bodies resembling empty spherules of coccidioides.

**Adrenal Gland** Foci of round cells and groups of pleomorphic cells were observed. There were groups of swollen vacuolated cortical cells, most abundant in the zona fasciculata. They did not contain fat and resembled the cells described by Miller and Kritzler.

**Liver** The hepatic cells contained considerable fat in large droplets, especially in the portal regions. The nodule seen grossly was composed of disorganized liver cords in which almost all the cells were fatty. Congestion, hemorrhage and agglutination of red cell masses in the sinusoids were noted within the nodule but not elsewhere in the liver.

**Other Organs** A scattering of large mononuclears was found in several places in the peripancreatic and intrapancreatic adipose tissue, the fat around the pelvis of the kidney, the periadrenal tissue, the breast and the interstitial tissue of the myocardium. In the heart were rare minute foci of fibrosis. A small thrombus partially filled the lumen of a renal vein. The spleen showed congestion and phagocytes containing red cells and hemosiderin. Marrow from several sites revealed no change other than a slight reduction in the number of myeloid elements and mild hemosiderosis.

Leukopenia was more pronounced in this case than in any of those previously reported, the lowest leukocyte count hitherto mentioned being 1,850.<sup>3</sup> The sedimentation rate was never ele-

vated despite severe infection and high fever. Although the ulceration of subcutaneous nodules which occurred is not usually present in cases of panniculitis, this feature has been described in an occasional report.

The patient obviously died of staphylococcal septicopyemia and not of panniculitis as such. The susceptibility to staphylococcal infection may be of no special significance, but staphylococci were also cultured from an ulcerated lesion in the Miller-Kritzler case. The repeatedly positive reactions to coccidioidin in cutaneous tests were explained by the healed primary lesion of coccidioidomycosis observed in the lung.

Despite the phagocytosis of red cells, lymphocytes and leukocytes by macrophages in the pannicular lesions and in the regional lymph nodes, there was surprisingly little stainable lipid material in the phagocytes. Few foam cells and no crystals were observed. Mitotic figures were abundant in the cells of the inflammatory exudate. The pseudoepitheliomatous hyperplasia of the epidermis at the edges of the ulcer on the thigh was far more extreme than is usually seen even in chronic ulcers of the legs.

The pathologic alterations in the viscera yielded no light as to the essential nature of the disease. Changes in visceral adipose tissue, such as those described by Spain and Foley,<sup>2c</sup> were not seen. It is not clear what significance, if any, may be attached to the focal lesions in the adrenal cortex observed in both the Miller-Kritzler and the present case.

In making a histologic diagnosis of panniculitis one must first distinguish between those conditions which involve the fat lobule only secondarily and those in which the adipose tissue is primarily affected.

Nonspecific inflammatory lesions, such as cellulitis, may extend into adjacent fat, but such a picture is rarely misinterpreted. Although erythema nodosum and erythema induratum may be characterized by exudative and granulomatous inflammation of the subcutaneous adipose tissue, the fibrous septums separating the fat lobules and the connective tissue of the dermis are involved as severely as the fat. Clearly defined tuberculoid granulomas and angitis are commonly seen in the erythemas but are not present in cases of panniculitis. In cases of true panniculitis, on the other hand, one is struck by the localization of the exudative and proliferative reaction to the fat lobule itself. The interlobular fibrous septums may be thickened, but they are relatively spared. It can be seen that the involvement of the dermis which does exist is due to inflammation of the adipose tissue about the appendages and accessory structures.

There is primary involvement of the fat lobules in a few conditions besides Weber-Christian disease. Traumatic fat necrosis may closely simulate primary panniculitis. In differentiating the two conditions the possibility that

3 Brill, I. C. Relapsing Febrile Nodular Non-suppurative Panniculitis (Weber-Christian Disease), in Medical Papers Dedicated to Henry Asbury Christian, Baltimore, Waverly Press, Inc., 1936, pp. 694-704.

one is dealing with a post-traumatic reaction can be excluded if multiple and recurrent lesions appear spontaneously and are unrelated to trauma. Although neonatal adiponecrosis<sup>4</sup> and some reactions to cold<sup>5</sup> may also resemble the lesions of panniculitis, usually they may be easily differentiated. However, in a recently studied case of "cold allergy",<sup>6</sup> a histologic appearance indistinguishable from that of Weber-Christian disease was encountered in a section of a nodule produced by the application of ice to the forearm.

Systemic diseases may involve the subcutaneous adipose tissue and result in apparent panniculitis. A case of Hodgkin's disease has been reported<sup>7</sup> in which the diagnosis of Weber-Christian disease was made at first because of the appearance of the cutaneous lesions. Panniculitis has also been noted in a case of Chagas' disease in which *Schizotrypanum cruzi* was

identified in the granulomatous lesions in the subcutaneous fat.<sup>8</sup>

It is recognized that the present case is not entirely typical of Weber-Christian disease as originally described. It probably should be grouped with other cases<sup>9</sup> in the literature which, while not altogether characteristic of the fully developed syndrome, are best considered as instances of primary panniculitis. Until more is known of the genesis of Weber-Christian disease, it might be well to broaden the diagnostic criteria and include in this category all cases in which primarily the subcutaneous fat lobule is involved by an idiopathic inflammatory process.

8 Mazza, S., Basso, G., and Basso, R. Investigaciones sobre enfermedad de Chagas. Comprobacion en adulto, de citoesteatonecrosis subcutanea chagastica por siembra hematogena (Chagomas hematogenos) de *S. cruzi*, Publication 48, Universidad de Buenos Aires, Mision de estudios de patologia regional argentina, 1940.

9 Rothmann, M. Virchows Arch f path Anat **136** 159, 1894. Llambias, J. Rev Soc argent de biol **6** 723, 1925. Abrikossoff, A. Centralbl f allg Path u path Anat **38** 542, 1926. Morone, G. Clin chir **32** 25, 1929. Carol, W. L. L., Prakken, J. R., and van Zwijndregt, H. A. Arch f Dermat u Syph **182** 329, 1941.

4 Fox, H. Arch Dermat & Syph **27** 237, 1933.

5 Hanthausen, H. Nord med (Hospital tid) **7** 1174, 1940. McGovern, T., Wright, I. S., and Kruger, E. Am Heart J **22** 583, 1941.

6 G. J. Heid and J. Fromer. Personal communication to the author.

7 Reimann, H. A., Havens, W. P., and Herbut, P. A. Arch Int Med **70** 434, 1942.

## TWO CASES OF CONGENITAL WEB OF A BRONCHUS

JAMES E. WALLACE, M.D., PITTSBURGH

The discovery of 2 cases in which emphysema in one lung and collapse in the other were associated with a small congenital web occluding the main bronchus on the collapsed side led to interest in the condition, for it appeared that if it could be diagnosed during life, the occluding membrane might be punctured and the condition completely cured.

Search of the literature failed to reveal any adequate reviews of the anomaly, in fact, I was able to find little on the subject.

### REPORT OF CASES

**CASE 1**—A 3 month old white girl was admitted to the Allegheny General Hospital from the Pittsburgh Home for Babies Feb 18, 1944, with a chief complaint of peculiar respirations.

It had been noticed that she had peculiar respirations since she was admitted to the Pittsburgh Home for Babies. She cried considerably before feeding and had difficulty with the mechanics of feeding. She was lethargic immediately after feeding. Roentgen examination Jan 28, 1944, at the Allegheny General Hospital showed complete atelectasis of the right lung. The left lung protruded into the right hemithorax, and the heart was on the right side of the chest. There were small congenital anomalies of the dorsal and cervical vertebrae.

There had been no nursing difficulties other than mechanical, and the child had been developing normally.

The examination showed a peculiar wheezing type of respiration, but there was no dyspnea. Breath sounds were reduced or absent on the right side and normal on the left. The cardiac point of maximum impulse was in the midline. There were no observable murmurs. The patient had a weak, high pitched cry. There was bilateral edema as well as bilateral cyanosis of the hands, the face and the lips, otherwise the child was essentially normal.

Bronchoscopy was done February 23 without the aid of anesthesia. A bronchoscope designed for use in newborn infants was introduced through a laryngoscope, similarly designed, into the trachea. A large amount of thick mucoid secretion was aspirated. This continued to be present in such amounts that definite landmarks could not be ascertained. After continuous aspiration of the discharge, the bronchoscope was removed without anything being observed that would suggest a diagnosis. The patient left the operating room in good condition but died February 25.

The autopsy revealed congenital atelectasis of the right lung, a congenital web of the right main bronchus between the superior and medial branch bronchi, hypertrophy of the left lung, compensatory emphysema, patchy atelectasis and hypostatic congestion of the left lung, mucopurulent bronchitis, dextroposition of the heart, a patent foramen ovale, a cystic right ovary.

The thymus measured 5 by 4 by 1.5 cm. It covered the entire right lung. The left lung was emphysematous and edematous. The heart was in the position normally occupied by the right lung, and the apex was

pointed dextrally. The heart, both arches of the aorta, the trachea, the bronchi and the lungs were removed en masse. The left lung measured 11 by 18 by 4 cm. There was a small area of atelectasis in the apex. Most of the lung was crepitant, but the base showed a marked amount of edema. The right lung measured 5 by 3 by 2 cm. It appeared to be solid except for a small air-containing bleb in the apex. The vocal cords were injected and slightly edematous. There was a mucous plug measuring 2 cm in length in the trachea.

The bronchus of the upper lobe of the right lung was visible and patent throughout. Immediately below the opening of this bronchus the main bronchus was completely occluded by a thin congenital web. On perforation of the web the bronchi to the middle and lower lobes could be seen. They were patent throughout. The bronchi to the left lung were injected and contained some mucopurulent material but were otherwise essentially normal.

The right ovary measured 2 by 2 by 1 cm and was made up of multiple small cysts. Otherwise the autopsy yielded essentially normal observations.

**CASE 2**—A Negro girl was born in Allegheny General Hospital by cesarean section. The routine respiratory stimulation was given, but the baby did not respond well. Her breathing was shallow and the cry weak and feeble. There was bilateral edema as well as cyanosis of the hands, the face and the lips. Plasma was given subcutaneously without the expected results. The baby was lethargic, continued to go downhill and died the following day.

The autopsy revealed congenital atelectasis of the left lung and congenital web of the left main bronchus, ecchymotic hemorrhages in the submucosa of the gastrointestinal tract, small multiple hepatic hemorrhages, ecchymotic hemorrhages in the submucosa of the bladder.

The left lung was considerably smaller than normal. The heart, the aorta, the trachea, both bronchi and both lungs were removed en masse. The heart and the aorta were dissected away and were found to be essentially normal. The trachea was apparently normal. On section the left main bronchus was closed by a thick fibrous congenital web about one-half way between the bifurcation of the trachea and the hilus of the left lung. The web was dense and hard. After the web was perforated, the remainder of the bronchial tree seemed to be normal. The left lung weighed 44 Gm and measured 5.5 by 5 by 2 cm. It was solid and sank immediately in water. The right lung weighed 43 Gm and measured 7 by 5 by 2 cm. It floated high in water and was crepitant throughout. The right lung was essentially normal.

The liver weighed 150 Gm and measured 12 by 8.5 by 3 cm. On section it was soft and showed many small hemorrhagic areas.

The gastrointestinal tract was normal except for punctate hemorrhages in the submucosa throughout its length. There were hemorrhages of a similar type in the mucosa of the urinary bladder.

Symptoms common to both cases were the weak cry, the constant crying before feeding, the lethargy after feeding, the wheezing respirations, the absence of breath sounds on the

affected side, the cardiac point of maximum impulse with deflection toward the affected side, the bilateral edema and cyanosis of the hands and feet, the edema and cyanosis of the lips and the difficulty with the mechanics of feeding

#### COMMENT

A congenital web may produce complete or almost complete occlusion and may occur either in the larynx or in one of the main bronchi. In either instance complete occlusion occurs when the membrane is imperforate, and the condition is then called total atresia. A congenital web is usually a thin membrane-like diaphragm, which is easily broken. The cause is unknown but is probably atavistic.

The laryngeal web is usually located at or near the glottic level, almost always at the anterior portions, uniting the two cords to a greater or a lesser extent. Posteriorly there is a somewhat crescentic margin of the airway. Infection of the pulmonary tract soon develops as a result of impaired action of the glottis in the hecic cycle.

The symptoms depend on the location of the web and the amount of occlusion. When the web is laryngeal and small in size, the only symptom may be a wheeze, so slight as to be overlooked until swelling of the glottic margins in intercurrent laryngitis increases the obstruction and causes dyspnea. The lesser webs may produce little interference with the laryngeal functions. Larger webs, on the contrary, may interfere with respiration, phonation, defense and hecic expulsion, bringing about dyspnea, stridor, hoarseness and severe pulmonary symptoms. Therefore the symptoms of partially occluding laryngeal webs may vary from a slight wheeze, apparent only on exertion, to those of obstructive laryngeal dyspnea leading to indrawing of the suprasternal notch and the epigastrium.

The infant with a completely occluding laryngeal web dies of asphyxia as a "blue baby", such patients are rarely seen except by the obstetrician.

Webs of the bronchi may be symptomless if confined to one side, but usually a wheeze is audible if the examiner's ear is placed close to the open mouth.

There is only one way of making the diagnosis during life and that is by looking at the interior of the larynx or of the bronchus. An attempt at diagnosing a web by inference

may jeopardize the life of the patient. When inferential methods are relied on the disease is often mistaken for asthma, congenital laryngeal stridor, diphtheria, posticus paralysis or laryngismus stridulus. If the patient is under 5 or 6 years of age, the only way a laryngeal examination can be made is by direct laryngoscopy, which will at once reveal a web if one is present. With older children and adults the mirror has been used, but these are the patients with smaller webs. Moreover, it is not always easy to differentiate between an anomaly and changes due to diphtheria, syphilis, tuberculosis, a foreign body or other postnatal cause.

Complications may occur as the result of any acute or chronic inflammatory disease, by increase of the stenosis itself or by failure to clear the tracheobronchial tree of secretions. This failure is due not only to the diminution of the lumen but to failure of glottic cooperation in the hecic cycle.

The congenital bronchial web is usually accompanied by a wheeze heard at the open mouth if the bronchial obstruction is partial, and by physical and roentgenographic signs if it is complete. The bronchoscope affords the only certain means of diagnosis.

A thin web of the larynx may be ruptured by passing the triangular dilator through the opening. Sometimes the simple passage of the bronchoscope may be sufficient. Perforation and bronchoscopic dilation will cure congenital bronchial web in most cases if done in infancy or early childhood.

#### SUMMARY

In the 2 cases of congenital web of the bronchus herein reported the diagnosis was made at autopsy. The malformation may not be as rare as the meager literature would indicate. Careful clinicians observing the signs and symptoms may be instrumental in maintaining the life and insuring better health of the patient by use of the bronchoscope.

The physician who discovers inequality in the size of the lungs, particularly if this is accompanied by cyanosis and edema of the lips, the hands and the feet, should examine the larynx and the main bronchi for possible congenital webs. The procedure if carried out routinely might serve to explain other obscure deaths in infants.

Bibliographic references will be found in author's reprints.

# Laboratory Methods and Technical Notes

## A NEW HEMOGLOBIN STAIN FOR HISTOLOGIC USE

### A Slightly Modified Van Gieson Stain

R C DUNN, M D

Surgeon, United States Public Health Service

AND

E C THOMPSON, M T

Senior Medical Technician, National Institute of Health

BETHESDA, MD

For some time there has been a great need for a good, reliable, nonfading routine tissue stain demonstrating hemoglobin. Ralph<sup>1</sup> described a stain making use of the benzidine-hemoglobin reaction which has been useful but which for several reasons did not fulfil our purpose. The brown color of the hemoglobin in Ralph's stain interfered with the visualization of the products of hemoglobin degeneration<sup>2</sup> so that a separate section had to be cut and stained for this purpose alone. For these reasons it seemed that a stain based on a slight modification of a routine method which gave to hemoglobin a bright distinctive color would be most desirable and practical.

In the course of experiments on the staining of hemoglobin casts in the kidneys of mice dying two to twenty-four hours after acute massive hemoglobinuria, just such a stain was developed by one of us (E C T). It was also found, by considerable trial and error, that after a short preliminary procedure requiring ten to twelve minutes the routine Van Gieson stain could be done on the same paraffin section without any of the valuable characteristics of either stain being lost. Therefore, if the Van Gieson stain is done routinely, no extra section is needed. No control slide is needed, as the erythrocytes in blood vessels also stain, they serve as a check on the stain and as a quick always available standard for colorimetric comparison.

#### STAINING PROCEDURE

- 1 Bring sections to water. Use a glass staining dish.
- 2 Stain in aqueous alum-hematoxylin<sup>3</sup> (0.25 per cent in 5 per cent alum) fifteen minutes.
- 3 Wash in tap water.

From the Pathology Laboratory (R C Dunn) and the Industrial Hygiene Research Laboratory (E C Thompson), National Institute of Health

- 1 Ralph, P H. *Stain Technol* **16** 105, 1941
- 2 Svrbely, J L, Dunn, R C, and Von Oettingen, W F. *J Indust Hyg & Toxicol* **26** 37, 1944
- 3 Mallory, F B. *Pathological Technique* Philadelphia, W B Saunders Company, 1938. See aqueous alum hematoxylin, p 70

4 Transfer to a 4 per cent aqueous solution of ferric ammonium sulfate (iron alum, violet crystals, reagent quality) for one minute.

5 Wash lightly in tap water.

6 Stain in aqueous alum-hematoxylin (0.25 per cent in 5 per cent alum) ten minutes.

7 Rinse quickly in tap water.

8 Stain in Van Gieson's solution<sup>4</sup> (13 cc 1 per cent acid fuchsin, 87 cc saturated aqueous picric acid [trinitrophenol] fifteen minutes).

9 Transfer directly to 95 per cent ethyl alcohol for three minutes. Agitate slides.

10 Transfer to absolute ethyl alcohol for three minutes.

11 Clear in xylene and mount in clarite.

*Fixation*—For routine use, neutral buffered formaldehyde solution is best, but in bone marrow and spleen the cell detail is better with Zenker's solution or Helly's fluid<sup>4a</sup>. Tissues must be fresh and well fixed. As with myelin, hematoxylin staining of hemoglobin appears to be impaired by prolonged standing in formaldehyde solutions. See later comment on Carnoy's fluid under staining of muscle.

*Interpretation of Staining*—Hemoglobin in red blood cells, hemoglobin casts in the kidney, fine and coarse globules of hemoglobin in reticuloendothelial cells of the spleen, the liver, the bone marrow and the epithelial cells of the convoluted tubules of the kidney all stain a green color that varies from a light olive green to deep pea green or blackish green, depending on the hemoglobin content. This color is fairly stable. In slides kept six months in the dark there was little fading.

Collagen stains red, as in the routine Van Gieson stain.

#### OBSERVATIONS AND COMMENT

During the examination of the organs of animals used in this study, other observations were made which may be of value to one using this stain.

With reference to casts in the renal tubules following massive hemoglobinuria, a few of the

4 Mallory, p 92. See Van Gieson's micro-acid fuchsin solution.

4a Helly's fluid is a modification of Zenker's solution in which, instead of 5 cc of glacial acetic acid, solution of formaldehyde U S P is added in the concentration of 5 per cent.

eosinophilic casts occasionally do not stain green but brown to brownish red. These casts will also not reveal hemoglobin after Ralph's benzidine stain. In this case we are convinced that no hemoglobin is present. However, in routine studies to determine toxicity in animals without hemoglobinuria, we have often found that ordinary eosinophilic hyaline casts are partially or completely green, indicating that some hemoglobin is present. "Old" hemoglobin casts, such as those observed in animals one to several weeks after poisoning, are often weakly eosinophilic and granular or globular. These are apparently degenerating and do not stain green like "fresh" hemoglobin, but light brownish yellow.

In the convoluted tubules of guinea pig kidneys we have repeatedly demonstrated species-specific triangular oxyhemoglobin crystals similar to those pictured as oxyhemoglobin crystals from guinea pig blood by Reichert and Brown.<sup>5</sup> This constitutes sufficient proof that the cast material in the same tubule is hemoglobin.

In the livers of animals with acute toxic hemoglobinuria a few to moderate numbers of hyperoxyphilic (eosin-methylene blue) hepatic cells will often stain deep green. Less often, eosinophilic hyaline globules in the cytoplasm of liver cells have been found to be green tinged, and epinuclear green staining of smooth muscle fibers in the portal arteries has also been noted.

Single and grouped fibers in the hearts of these animals often stain green. They occasionally resemble in appearance and location the Purkinje fibers described and pictured by Todd.<sup>6</sup>

5 Reichert, E. T., and Brown, A. The Crystallography of Hemoglobins, Publication 16, Carnegie Institute of Washington, 1909.

6 Todd, T. W., in Cowdry, E. V. Special Cytology, New York, Paul B. Hoeber, Inc., 1932, vol. 2, pp. 1175-1210.

Other green-stained cells, single or grouped, occasionally interconnected, are often seen in the ventricular myocardium. A few to many of these cells were seen in routine sections from all but a few animals with acute hemoglobinuria. That Purkinje cells should stain is not surprising, since Todd recommended the iron hematoxylin procedure, which is the essential basis for the hemoglobin stain. If the green staining of these fibers is due to hemoglobin taken up from the blood, how does the cell take up this pigment? Of course, the cell wall may be injured, but according to Todd, "It would seem that the Purkinje element is endowed with phagocytic properties" for disintegrating leukocytes have been observed in Purkinje cells. Another question that most certainly will occur to many is whether or not green staining indicates damage to the cell.

Todd inferred that the study of Purkinje cells in normal human hearts is difficult, while in some hearts from persons with pathologic states (pneumonia, syphilis) they are studied and stained with comparative ease. In fact, he says it is necessary to study the abnormal heart to understand the normal Purkinje system. Since we have never yet seen green-stained fibers in the heart of a normal animal, there is a strong possibility that green staining is indicative of damage.

As yet we have studied skeletal muscle in only a small series of animals. However, while we have never observed green-stained fibers in normal muscle fixed in solution of formaldehyde, we have observed them in muscle showing evidence of damage following fixation in Carnoy's fluid. The possible relationship between green staining and the status of myoglobin in the cell has not been determined.

# General Reviews

## ARTERIOSCLEROSIS

W C HUEPER, M D  
NEW YORK

### THE ANOXEMIA THEORY

(Continued from Page 364)

#### INTRAVASCULAR HYDROSTATIC PRESSURE A DECREASED

The second main group of arteriosclerogenic agents which elicit changes in the blood pressure comprises those which exert their hypotensive or hypertensive action through causing variations in the degree of filling of the vascular lumens with blood and thus influence the intravascular hydrostatic pressure. Mention of the action of this arteriosclerogenic mechanism as a complicating factor was made in the preceding discussions of the vascular lesions observed in connection with several hypertonic types of arteriosclerosis (thromboangitis obliterans, Raynaud's disease, and renal and essential hypertension).

Hydrostatic and hydrodynamic factors which reduce the intravascular pressure by decreasing the amount of blood delivered into a particular part of the vascular system produce a local or a general hypotensive state in small or in large portions of the vascular bed. Hence the vascular bed becomes relatively too large for the amount of blood available in spite of maximum contraction of the muscular media. There ensues a reduction in blood supply and in pulse pressure which in turn results in ischemic anoxemia of the vascular walls. Under such conditions the main functional strain rests on the muscular elements, while the elastic components of the wall are relaxed and suffer rather from disuse if the causal conditions are of long duration.

Decreased intravascular hydrostatic pressure is the result of physiologic and pathologic factors. The physiologic mechanism of disuse of certain parts of the vascular system becomes operative when the circulation changes after birth from the fetal, umbilical type to the postnatal, pulmonary type, and it is active in the uterus and the ovaries in connection with the physiologic involutionary changes of the female gonads after menstruation, pregnancy and especially the menopause. Pathologic factors of this type affect the

distal portions of ligated or thrombosed vessels, those parts of the arterial bed distal from arteriovenous aneurysms or from intra-arterial obstructions or stenoses, and arteries located in scar tissue.

**ENDOGENOUS MECHANISMS—*Postnatal Involutionary Sclerosis of the Arteries of the Umbilical Circulation***—When, with the beginning of the respiratory movements after birth, the pulmonary circulation is opened, the pulmonary artery no longer sends its blood through the ductus arteriosus Botalli into the aorta but delivers it into the pulmonary vessels. As a result of this change in the circulatory conditions, the ductus arteriosus collapses, and the pressure in the aorta, which after this event receives blood only from the left ventricle, becomes insufficient to press blood into the umbilical arteries (Thoma, Ranke). The ductus arteriosus and the umbilical arteries therefore contract, undergo atrophic changes on the basis of disuse and usually obliterate within the course of the first few months of life.

With the complete or almost complete disappearance of the intravascular hydrostatic pressure there ensues in the ductus arteriosus a fibrous proliferation of the intima and the media associated with a simultaneous atrophic degeneration of the muscle cells (Bell, Benda, Kaufmann, MacCallum). Pfeifer reported an increase in the medial elastic tissue accompanying these changes. The lumen of the collapsed vessel finally becomes occluded by fibrous tissue. The elastic elements in the media disintegrate gradually, and fibrohyaline scar tissue takes the place of the original vessel.

Similar changes occur in the umbilical arteries, whose structure resembles that of the uterine arteries. The umbilical arteries contract after the ligation of the umbilical cord. On the second day thereafter a focal swelling of the endothelial cells develops, followed by the appearance of

---

From the Warner Institute of Therapeutic Research



fibrinoid deposits. A few days later fatty dust appears in the subendothelial layer and in the swollen muscular media. With the subsequent necrosis of the media, the vascular lumen becomes dilated. The internal elastic membrane also undergoes swelling and homogenization. From the third week on fibroblasts appear, which proliferate into the plasmatic matter filling the lumen. This filling tissue becomes vascularized by formation of capillaries. Following gradual disappearance of the latter a fibrohyaline tissue is left, which also takes the place of the degenerated and sometimes calcified media.

In cases in which the umbilical arteries do not become obliterated, a loose fibroblastic tissue is formed between the endothelium and the internal elastic membrane. Marked elastosis, which is similar to the lamellated elastosis seen in renal vessels of nephrosclerotic kidneys, then appears between the longitudinal muscle bundles, which undergo gradual degeneration and replacement by fibrous tissue (Schallock, Kaufmann, Pfeifer, Thoma). Finally a new internal elastic membrane may develop beneath the endothelium. These changes resemble those seen in the uterine arteries during senile involution, in arteries in the environment of chronic gastric ulcers and in endarteritis obliterans.

Occasionally the obliterating type of involution of the umbilical arteries is complicated by the appearance of a diffuse serous imbibition of the entire wall, sometimes associated with precipitation of fibrinoid material and infiltration of leukocytes. These changes have a definite similarity to the arterionecrotic and necrotizing arteriolitic lesions observed in periarteritis nodosa, rheumatic arteritis, malignant nephrosclerosis and accelerated glomerulonephritis (Schallock).

The different types of reactions described reflect to a certain degree the various arteriosclerogenic mechanisms active in their production. The obliterative endarteritic changes with atrophy and fibrosis of the media represent the vascular reaction to decreased intravascular hydrostatic pressure, as pointed out many years ago by Thoma, who stated that only the blood flow protects the arteries from obliteration. In the nonobliterating elastotic type of reaction the primary hypotensive mechanism is complicated by a secondary compensatory elastotic reaction developing in response to the atrophy of the muscular elements. The elasticity and the distensibility of the wall of the patent umbilical arteries then depend entirely on the proliferated elastic elements.

In the third type of reaction reported, secondary vasotoxic and probably allergic influ-

ences are active and superimposed on the endarteritic changes.

*Involutionary Sclerosis of the Uterine and Ovarian Arteries*—Another and outstanding example of the arteriosclerogenic effect of local hydrostatic hypotension is offered by the vascular sclerosis observed in the uterine and ovarian arteries as the result of mild to severe fluctuations in the circulatory and hemodynamic conditions occurring in these organs in connection with menstrual periods, pregnancies and the menopause (Ranke, Hueck, Jacob, Westphalen, Bennecke). It is for this reason that the uterine arteries show early and frequent sclerotic intimal changes (Kon and Karaki, Ditttrich).

The intima of the uterine arteries, which is thin during the first decade of life, increases in thickness by fibroblastic proliferation during the latter half of the second decade, i. e., after the onset of puberty and of rhythmic menstrual fluctuations of increasing and decreasing blood supply of the uterus. These changes affect mainly the large arteries in the outer and the middle portion of this organ and produce an eccentric shift of the lumens. During the third decade small hyaline foci are occasionally seen in the thickened intima. These proliferative and degenerative reactions become much more pronounced in incidence and degree during the fourth decade and involve also the medium-sized arteries. The intimal changes are still predominantly fibrous at this time and only occasionally hyaline. There also occur during this period hyaline degenerations in the media, particularly in that of the medium-sized arteries. In the subsequent decades the arterial lesions show a progressive character. Calcifications in the intima and the media are seen together with extensive intimal and medial hyalinization. The arteriolar walls become markedly swollen and hyaline. Kon and Karaki did not find any consistent relations between the severity of these lesions and the occurrence and the number of pregnancies, as the same type and degree of lesions were sometimes observed in women who had not been pregnant.

After the onset of the menopause, when the rhythmic fluctuations of the filling of the uterine vessels synchronous with the menstrual periods cease, the changes of senile involution are superimposed on the preceding effects of menstruation and pregnancy. The intima then shows fibroelastic thickening accompanied by a considerable increase of the mucoid ground substance with hyalinosis and calcinosis, both of which extend into the atrophic, fibrous and hyaline media (Fraenkel, Meyer, Pankow). The demarcation between the intima and the media gradually dis-

appears and the lumen becomes increasingly narrowed. The process was designated by some investigators as endarteritis obliterans (Reincke, von Kahlen, Balin, Dittrich, Meyer, Wiegand, Palmer). In histochemical studies Zinkant, who used ashed sections of the uteri of various age groups, showed that the deposition of calcium starts with the second decade and is regular from the third decade, involving at this time only the intima. From the fifth decade on, calcifications appear also in the media and are then observed also in the smallest arteries, particularly in the elastic layers.

The puerperal changes differ from the pre-senile and the senile ones, since they develop relatively rapidly as acute involutionary reactions of the hyperplastic and dilated uterine vessels of pregnancy. During pregnancy there appear in the intima cushion-like connective tissue thickenings, with deposition of a metachromatic mucoid substance, and a proliferation of elastic fibrils sometimes forming several lamellas (Wermbter, Pick, Woltke). With succeeding pregnancies, these changes become more pronounced, and the metachromatic material and the elastic lamellas appear also in the perivascular tissue. After delivery regressive changes set in in the contracted uterine vessels, which, moreover, are compressed from the outside by the firmly contracted uterus (LaTorre). The lumens of the uterine arteries become narrowed by a considerable fibrous proliferation of the intima, which extends also into the media and which may lead to complete obliteration of the vessel. The muscular elements of the media undergo simultaneously fatty degeneration and finally complete dissolution affecting either the entire media or only part of it while another part is replaced by fibrous tissue (Balin, Broers, Woltke, Mayor, Kon and Karaki, Frankl and Stolpe, Buttner, Pankow, Wermbter, Goodall).

The ovarian arteries pass through similar endarteritic changes, which start relatively early in life, especially in the neighborhood of corpora fibrosa. The proliferation of elastic lamellas in the intima of the ovarian arteries is associated with fibrous atrophy of the muscularis. Calcifications appear in the border zone between the intima and the media (Woltke, Borchardt, Westphalen).

The sclerotic lesions of the uterine and ovarian arteries occur independently of those developing in the arteries of the rest of the body and illustrate, therefore, the importance of local functional factors in the production of such changes. It is obvious that during the time of active sexual function the vessels of the uterus and the ovaries

pass through periods of increased intravascular hydrostatic pressure, accounting for the presence of elastic proliferation and mucohyaline material in the intima, followed by periods of decreased intravascular hydrostatic pressure, which elicit the endarteritic and medial fibrosing reactions. These hydrodynamic conditions become persistent after the menopause, leading to an accentuation of the endarteritic responses.

**EXOGENOUS MECHANISMS—*Arterial Occlusion, Stenosis and Compression***—A reduction in intravascular hydrostatic pressure follows partial or complete occlusion of an arterial lumen by thrombi or atheromas, pressure from the outside of scar tissue, tumors or other structures, kinking or torsion of the vessel and ligation. Mention was made, in connection with the discussion of the various vascular changes associated with thromboangitis obliterans, of the occurrence of endarteritic lesions in the small arteries of the extremities distal to the narrowing of the vascular lumens of large arteries by mural thrombi and marked intimal fibrous thickenings. Marked and finally obliterating fibroblastic intimal proliferations accompanied by atrophy and fibrosis of the muscular media are found in parts of arteries located distal from ligations and not supplied by blood from anastomosing vessels. Malyschew, Apollonio and Peckelharing studied these changes in dogs and rabbits in which the carotid or crural arteries were ligated. If the blood was expressed from the vascular lumen before a double ligation was made, the endothelial cells proliferated into the collapsed lumen within seven to ten days, with numerous mitoses present in these cells. If blood was left in the ligated vessel, endothelial cells emigrated into the blood clot in a fashion similar to that of fibroblasts in tissue cultures. Myelopoietic foci were then formed in the organizing blood clot, which was finally converted under formation of vascular lumens into an angiomatous structure (Malyschew). Peckelharing concluded from these observations that it is the removal of intravascular pressure which elicits in collapsed ligated vessels the endarteritic reaction.

The same hydrostatic mechanism is active in the production of the fibrous intimal thickenings and the narrowing of the arterial lumens in the stumps of amputated extremities, as the caliber of such arteries has become too large for the amount of blood needed by the reduced areas of tissue (Ranke, Muller). Similar ischemic fibrosing intimal changes are seen in the arteries distal from arteriovenous fistulas (Mahrburg), where the intravascular hydrostatic pressure drops sharply.

Vasospasm and mechanical compression or partial occlusion of the lumens of proximal arteries resulting in a decrease of the intravascular hydrostatic pressure and of the pulse pressure are responsible for the arteriolar endarteritis recorded present in kidneys with chronic glomerulonephritis and essential nephrosclerosis. Cicatricial changes in the perivascular connective tissue likewise cause sclerosing lesions in the small arteries of the skin and other organs in scleroderma (Heine, Murphy, Kramin and Gerson, Goldman, and others), in chronic radio-dermatitis and in the floor of roentgen ulcers (Warren, Hueper, Hueper, Fisher, de Carvajal-Forero and Thompson, and others) and of chronic gastric ulcers (Torhorst, Hueper and Ichniowski, Meyer and Saphir, Fetterman, Buday, Lewin)

#### INTRAVASCULAR HYDROSTATIC PRESSURE A DECREASED

- Apollonio, C Beitr z path Anat u z allg Path **3** 290, 1888  
 Baln, J Arch f Gynak **15** 157, 1879  
 Bell, E T Arteriosclerosis of the Abdominal Viscera and Extremities, in Cowdry, E V Arteriosclerosis, New York, The Macmillan Company, 1933, p 473  
 Benda, C Die Gefasse, in Aschoff, L Pathologische Anatomie, ed 4, Jena, G Fischer, 1919, vol 2, p 71  
 Bennecke, A Virchows Arch f path Anat **191** 226, 1908  
 Borchardt, H Virchows Arch f path Anat **259** 373, 1926  
 Broers, C W Virchows Arch f path Anat **141** 72, 1895  
 Buday, K V Beitr z path Anat u z allg Path **44** 327, 1928  
 Dittrich, P Ztschr f Heilk **10** 15, 1889  
 Fetterman, G H Arch Path **20** 189, 1935  
 Goldman, D Arch Int Med **70** 822, 1942  
 Heine, J Virchows Arch f path Anat **262** 351, 1926  
 Hueper, W C Occupational Tumors and Allied Diseases, Springfield, Ill, Charles C Thomas, Publisher, 1942  
 —and Ichniowski, C T Am J Pharmacol & Exper Therap **78** 127, 1943  
 —Fisher, C V, de Carvajal-Forero, J, and Thompson, M R J Urol **47** 156, 1942  
 von Kahlen, C Beitr z path Anat u z allg Path **23** 161, 1898  
 Kaufmann, E Lehrbuch der speziellen pathologischen Anatomie, ed 7, Leipzig, Vereinigung wissenschaftlicher Verleger, 1922, vol 1, p 71  
 Kon, J, and Karaki, Y Virchows Arch f path Anat **191** 456, 1908  
 Lewin, A M Arch f Verdauungskr **14** 114, 1908  
 MacCallum, W G A Text-Book of Pathology, ed 4, Philadelphia, W B Saunders Company, 1928, p 331  
 Mahrburg, S Virchows Arch f path Anat **274** 528, 1929  
 Malyschew, B F Virchows Arch f path Anat **272** 727, 1929  
 Mavor, A Ann d physiol **3** 560, 1887

- Meyer, J, and Saphir, O Am J Digest Dis **10** 28, 1943  
 Muller, H Verhandl d deutsch path Gesellsch **19** 307, 1923  
 Murphy, J R, Kramin, P, and Gerson, M J J A M A **116** 499, 1941  
 Palmer Am J Obst & Gynec **43** 30, 1898  
 Pankow Arch f Gynak **80** 271, 1906  
 Peckelharig, C A Beitr z path Anat u z allg Path **8** 245, 1890  
 Pfeifer Virchows Arch f path Anat **167** 210, 1902  
 Ranke, O Beitr z path Anat u z allg Path **75** 269, 1926  
 Reinecke Arch f Gynak **53** 340, 1897  
 Schallock, G Virchows Arch f path Anat **302** 195, 1938  
 Sohma Arch f Gynak **84** 377, 1908  
 Thoma, R Virchows Arch f path Anat **204** 1, 1911, **93** 443, 1883, Beitr z path Anat u z allg Path **10** 433, 1891  
 Torhorst, A Beitr z path Anat u z allg Path **95** 489, 1935  
 Warren, S Arch Path **34** 443, 562, 749, 917 and 1070, 1942, **35** 121 and 304, 1943  
 Werbter, F Virchows Arch f path Anat **257** 249, 1925  
 Westphalen, H Virchows Arch f path Anat **106** 420, 1886  
 Woltke, W Beitr z path Anat u z allg Path **27** 575, 1900  
 Zinkant, W Virchows Arch f path Anat **281** 911, 1931

#### INTRAVASCULAR HYDROSTATIC PRESSURE B INCREASED

Increased intravascular hydrostatic pressure causing hydrostatic hypertension results from the presence of an excessive amount of blood in the entire circulatory system or in parts of it. The vascular walls are thereby mechanically distended beyond the limits of their normal capacity, and both the muscular and the elastic elements are stretched and placed in a state of accentuated functional activity. Moreover, the contractile and elastic components are pressed against the unyielding adventitia, causing compression of the vasa vasorum, which, in addition, are elongated and thus partially collapsed by being longitudinally stretched. Ischemic anoxemia is thus produced in the vascular walls by hydrostatic hypertension.

This condition when affecting the entire circulatory system (plethora) is either of endogenous origin (polyhemia or polycythemia) or of exogenous genesis (a habitual excessive introduction of watery liquids). Locally increased hydrostatic pressure results from abnormalities in the heart or the vascular system, such as malformations of the heart or of the large elastic arteries, from arteriovenous fistulas, from extraordinary local functional demands for blood or from highly accentuated forces of gravity causing delivery or shift of excessive amounts of blood to restricted areas of the arterial network.

**ENDOGENOUS MECHANISMS**—*General Hydrostatic Hypertension or Plethora*—Moschcowitz once advanced the axiom that without increased intravascular pressure there is no arteriosclerosis. However, a general causative role of hydrostatic hypertension in the production of the generalized proliferative and degenerative vascular changes known as arteriosclerosis cannot be recognized, as there occur many cases of arteriosclerosis without hypertension of any kind at any time and even the presence of polyhemia, or plethora, in many hypertensive conditions is controversial. While some (Aschoff, Plesch) have favored such a relationship, others (Jaffé, Dietrich, Griesbach) have doubted the reliability of the evidence on which this conclusion is based. Some (Buengeler, Kahler, Volhard, Baucke) have maintained that plethora exists in red, or benign, hypertension, but others (Muller, Ernst, Stageschmid, Schmid) have contradicted this statement. The total blood volume, on the other hand, seems to be low in pale, or essential, and glomerulonephritic types of hypertension and in hypertension of the Goldblatt type (Buengeler, Griffith, Rutherford, Roberts and Lindauer, Beckwith and Chanutin). The hypertension found with thyrotoxicosis has been attributed to plethora combined with increased minute volume of the heart (Volhard). It was reported by Ruggiero that the plethora associated with polycythemia vera is accompanied by a relatively high incidence of degenerative vascular lesions. Plesch claimed that status plethoricus leads rapidly to arteriosclerosis. However, the actual role and significance of these endogenous types of plethora in the production of arteriosclerosis are not yet definitely established.

In addition to these often erythrocytemic types of plethora there is an exogenous type elicited by habitual and rapid consumption of excessive amounts of liquids usually in the form of beer or wine (Rossle, Ernst, Flaig, Heubner, Genersich). The so-called hypertrophic "beer heart" is attributed to the hemodynamic conditions connected with this artificial exogenous plethora. Israel found in the aorta and the large elastic arteries of drunkards a marked reduction of elasticity and the smallest degree of distensibility of all arteries studied. Causal relations of the high incidence of arteriosclerosis, arteriosclerosis and endarteritis among drunkards to the overindulgence in beer and wine have been claimed by Koelsch, Heubner, Guttmann, Flaig and Genersich. It may be added that in man and animals the introduction of excessive amounts of water for experimental purposes has resulted in a marked increase of the blood pres-

sure (Miller and Williams, Rowntree, Griffith, Griffith, Rutherford, Roberts and Lindauer). Chronic experiments of this type for the determination of any potential vascular effects have apparently not been made.

In this connection it may be mentioned briefly that any arteriosclerogenic changes allegedly caused by excessive consumption of alcoholic beverages cannot be blamed on a direct effect of the alcohol on the vessels or on their tonus, as has been asserted (Hultgren). The studies of numerous investigators (Cabot, Leary, Martland, Fahr, Eberhard, Gorog) on persons indulging mainly in the consumption of hard liquor showed that these exhibited a relatively low degree of arteriosclerosis. Ruffer, on the other hand, stated that Mohammedans, who are exceptionally strict in their total abstinence from alcohol, have arteriosclerosis as often as Europeans. The studies of a number of workers cited by Schirokogoroff (Afanassieff and Kremjanski, Kulbin, Bondareff, Lebenson, Tepljaschin) on rabbits, dogs, guinea pigs and rats receiving alcohol orally for up to nine months were inconclusive, only a few small atheromatous thickenings near aortic valves were observed in some of the dogs used by Afanassieff. Negative results in animals after chronic feeding of alcohol were reported by Petrov and by Fahı. Saltykow injected alcohol intravenously into young rabbits eight times during a period of two years and observed only in 1 severe intimal thickenings of the aorta, hepatic arteries and renal arteries.

*Local Hydrostatic Hypertension*—(a) In Collateral Arteries after Ligation or Stenosis of a Main Vessel. When a main vessel is ligated or its lumen narrowed, the collateral vessels above this point are forced to take over the distribution of at least a part of the amount of blood originally passing through the occluded artery. From this hemodynamic circumstance they are exposed to an elevation of their normal intravascular hydrostatic pressure. Nothnagel observed in man and Jores and Schilling in rabbits and a dog subjected to these conditions endothelial proliferation and intimal hyperplasia of the small collateral arteries of an endarteritic type.

(b) In an Arteriovenous Fistula. Similar hemodynamic conditions prevail in the venous part of an arteriovenous fistula, which when affecting larger vessels short-circuits the circulation and increases the return flow of blood to the heart, causing general hypertension through an increase of the minute volume of the heart. The part of the vein proximal to the arteriovenous fistula undergoes structural arterialization. Associated with these hypertrophic changes

are calcification, thickening and fibrosis of the media and fibroelastic proliferation of the intima opposite the fistulous opening (Reid, Holman, Mahrburg, Callander, Dandy, Gamm, Weaver) in response to the marked local increase of intravascular hydrostatic pressure

(c) *At Bifurcations* The presence of whorls at bifurcations and the directional influence exerted by the vascular spurs of bifurcations on the flow of the blood are responsible for the increased intravascular pressure on certain adjacent parts of the vascular walls. Thus Chiari reported the occurrence in juveniles of a stripe-shaped fibrous endarteritic lesion at the bifurcation of the common carotid artery, extending from this vessel into the internal carotid artery. Similar observations at this location were reported by Beneke. Des Ligneis found ribbon-like white fibrous intimal thickenings on the posterior wall of the external iliac artery, extending to the orifice of the femoral artery. Such lesions were noted in 33 per cent of 100 consecutive autopsies. They were attributed to the mechanical impact of the blood on the posterior wall of this vessel occurring when the legs are bent and the artery is stretched. It is uncertain whether the intimal fibrous and elastic thickenings observed at bifurcations of cerebral arteries are attributable to the same hydrostatic mechanism or represent in part compensatory hyperplasia of the intima on the basis of congenital local medial defects (Voncken, Wallesch, Lowenhardt, Hey, von Hofman, Lebert, Tut-hill, Hackel, Beneke, and others)

(d) *With Coarctation of the Aorta* Coarctation or stenosis of the aorta is associated with mechanically conditioned hydrostatic hypertension in the circulatory system supplying the upper half of the body, particularly the upper extremities, and with normal or reduced blood pressure in the lower half of the body (Brotchner). The arteries of the upper half of the body, including the proximal portion of the aorta, are dilated because of the back pressure exerted on the aortic blood flow by the narrowing in the aortic lumen (Kohn, Irvine, Baker and Shelden, Sella). In many of the cases reported there were marked intimal thickenings and medial fibroses and calcifications in the aorta and the branches of the aorta above the stenosis (coronary arteries) as well as in the pulmonary arteries (Benkwitz and Hunter, Hickl, Gerhartz, Lemon, Read and Krumbhaar, Harrison, Evans, Legg). Cystic degeneration of the ascending aorta with spontaneous rupture of the vessel in connection with aortic coarctation was reported by Harrison and by Sella, who collected 10 additional cases from

the literature. In some of the patients, many of whom were juveniles or young adults, there were also some sclerotic changes below the stenosis or even definite renal sclerotic lesions. It is obvious that stenosis at the usual location in the upper part of the aorta impairs the blood supply to the kidneys and may thus elicit renal ischemic hypertension in cases in which a critical anoxemic level in the renal blood is surpassed. Experimental constrictions of the aorta in animals rendered similar results. Harvey, who transiently compressed the abdominal aorta of the rabbit below the renal arterial orifices, obtained temporary hypertension. After repeated compressions he observed primary medial necroses, fibroses and calcifications in the ascending and thoracic portions of the aorta. Dill and Isenhour performed similar experiments on rabbits maintained on a cholesterol diet but constricted the abdominal portion of the aorta proximal or distal to the orifices of the renal arteries. They observed intimal thickenings of the ascending and thoracic portions of the aorta composed of histiocytic cells and amorphous material containing cholesterol clefts, and duplication of the elastic membrane.

In this connection attention may finally be called to the comparatively high frequency of hypertension and of obliterating endarteritis in persons who have undergone leg amputations, as a hydrostatic factor possibly contributes to the development of these functional and anatomic abnormalities (Groedel).

**EXOGENOUS MECHANISMS**—*Local Hydrostatic Hypertension*—(a) *Physical Labor and Static and Gravitational Factors* Heavy muscular labor causes dilatation of the arteries supplying the involved muscles with blood, as shown by Schretzenmayr by oncometric methods in connection with the femoral artery. This reaction is attributable to the fact that the accentuated metabolism of working muscles (a three to eight fold increase of metabolic rate) increases the demand for blood (Ivy). During exercise there occurs, therefore, an increase in the total volume of the circulating blood as a result of the mobilization of blood from the depots (Simonson and Enzer). The margin of safety of cardiovascular function is reduced during work and may completely disappear when labor is excessive and prolonged. Under such circumstances metabolic exhaustion with relative anoxemia may ensue causing myocardial necroses of the heart (Froboese, Buchner and von Lucadou).

It is apparently from these causes (excessive vascular dilatation with increased hydrostatic intravascular pressure and metabolic insuffi-

ciency) that physically hard-working persons allegedly show a precocious appearance and a more marked development of arteriosclerosis of the arteries of the extremities than men subjected to less physical labor. Klotz stated that this type of work arteriosclerosis involves usually the right radial artery more severely than the left in right-handed persons, while these relations are reversed in left-handed persons. Similar observations were reported by Boveri. Kulbs commented on the high incidence of arteriosclerosis of the arteries of the arms in manual laborers, particularly paviors. Koelsch and Lederer made analogous observations in workers of the plate glass industry and of tanneries, where they are subject to strenuous manual labor. Hardened radial arteries were found among rather young persons of these occupations and after only ten years of work in such operations (Weissgerber). Britten and Thompson, studying 10,062 industrial workers, noted a prevalence of radial arterial thickening among industrial workers.

Identical conditions apparently prevail in workers using mainly their legs and suffering therefore from precocious arteriosclerosis of the femoral arteries. Kazda noted that the arteries of the leg which is exercised most (the work leg) show the most pronounced arteriosclerotic changes. A similar observation was reported by Heusner, who observed that in persons having only one well functioning leg (e. g., after infantile paralysis) the arteries of this leg exhibit sclerotic lesions earlier and more extensively than those of the leg with impaired function. Professional bicyclists, who continuously use their legs to an excessive degree, are said to suffer from early arteriosclerosis of the arteries of the legs as the result of increased regional intravascular hydrostatic pressure, but Heusner expressed doubt that this contention is correct. However, recent observations of Lake, Platt and Wright among employees of a large department store provide some support for such interrelations between strenuous occupational use of the legs and the development of precocious arteriosclerosis of the arteries of the legs. These investigators noted that the incidence of arteriosclerotic medial calcifications in the legs as demonstrated by roentgenographic methods was approximately twice as high in stair climbers (54 per cent) as in those who did not climb stairs (27 per cent) in the age group 40 to 49 years. Sternberg, according to Miller, found among members of occupations calling for an undue amount and degree of specialized physical activity, as weavers, who use their lower limbs extensively, a preponderance of arteriosclerosis of the vessels of these parts.

Miller cited Cordier as calling attention to arterial changes and hypertension in French soldiers undergoing much marching.

The increase in the intravascular hydrostatic pressure in the arteries of the lower extremities may result not only from excessive physical strain but from prolonged standing, as in this position the cardiovascular adaptation becomes defective and a circulatory deficiency results causing, under the influence of gravity, an accumulation of the blood in the dependent parts. Investigations made by Keys and Butt have shown that the vessels of the legs undergo changes in their permeability on quiet standing, as hemoconcentration occurs by transudation of fluid to the tissue spaces of the extremities. This process is accompanied by a marked increase in the colloid osmotic pressure of the serum (58 per cent) and a rise in plasma proteins, cholesterol, fatty acids and lipid phosphorus (from 21 to 24 per cent). Aschoff proposed that the increase of hydrostatic pressure in the arteries of the legs with these static conditions may be related to the medial calcifications often found in these arteries, according to Monckeberg.

Klotz attempted to imitate this arteriosclerogenic effect of static factors by suspending rabbits by the hindlegs for short periods daily. While he observed in the ascending and the thoracic portions of the aorta and the subclavical, brachial and carotid arteries of these animals medial degenerations and calcifications and intimal fibroelastic thickenings, subsequent investigators (Fahr, Lubarsch, Saltykow, Steinbiss) failed to confirm his observations.

Inasmuch as the arteries of the extremities are of the muscular type, the main functional and metabolic stress of withstanding prolonged excessive intravascular pressure of orthostatic genesis rests with the contractile elements. Muscle cells are not properly adapted for such a sustained strain, as this function belongs to the elastic elements. It is therefore plausible that under such conditions the muscle cells should undergo degenerative changes ending in local or diffuse calcinosis. The erect posture of man accounts for the fact that this orthostatic stress is in general more frequent and severe in the arteries of the legs than in those of the arms. It is apparently mainly from this fact that medio-calcinosis occurs much more frequently and extensively in the arteries of the lower than in those of the upper limbs (Cali).

In this connection attention must be called to the marked fluctuations in intravascular hydrostatic pressure occurring in airplane pilots during acrobatic flying, as a result of acceleration



(Adams) These accentuations of the forces of gravity may act centrifugally as well as centripetally and thus may cause local hydrostatic hypertension as well as local hydrostatic hypotension. During power dives and in narrow curves the hydrostatic pressure may become almost zero in some parts and may reach in other parts, on the other hand, unbelievably high figures (Ranke). Depending on the direction of the forces of acceleration, the temporary circulatory collapse may be accompanied by a lack of blood in the cerebral vessels or by an overdistention of the cerebral vessels with blood.

Such gravitational stresses of a hydrostatic nature on the walls of the blood vessels of flyers become especially marked during abrupt changes in the direction of movement, as in reversing the direction after a power dive, when these forces may reach values of up to 7.8 to 10.5 g (gravity). They are exerted mainly on the smaller vessels during such sudden and massive shifts of large amounts of blood from the caudal to the cranial parts or vice versa. Simultaneously there occurs severe interference with the return flow of blood to the heart and with the coronary circulation (Jongbloed and Noyons, Adams, Jokl, Poppen, Cross and Ball). Only suggestive information is as yet available as to the anatomic effects on the vascular system of often repeated hydrostatic episodes of this type. In a future evaluation of the evidence of cardiovascular pathologic changes in flying, however, attention must be paid to the fact that the hydrostatic aspect is probably often complicated by the effects of additional arteriosclerotogenic factors likely to be active in flyers, such as atmospheric anoxemia, poisoning with carbon monoxide, oxygen and lead, and exposure to cold. It is known from the sudden experimental increases of the hydrostatic intravascular pressure accomplished when large amounts of liquids have been injected under pressure into the vascular system of animals that tears occur in the elastic fibrils, followed by degeneration and calcification of the media and fibrous thickening of the intima (Malkoff, Ziegler, Ernst).

(b) Compression of Splenic Arterioles. Anomalous Condition of Testicular Arterioles. The vessels of the spleen are subject to marked fluctuation in their blood content and thus in their intravascular hydrostatic pressure, depending on the state of contraction of the splenic pulp (Westphalen). The human spleen can retain about 200 cc or one twentieth to one thirtieth of the total amount of blood while the canine spleen can retain up to one fifth and that of the cat up to one seventh (Steinmann, Eppinger). It is characteristic of the small arteries of the spleen

that there begins rather early in life and apart from any changes in other vessels a subendothelial deposition of a hyaline matter which causes thickening of the wall and narrowing of the lumen (Herxheimer, Matsuno, Nakonet-schny, Staemmler, Jacob). The hyaline lesions thus produced are progressive with age and become complicated by regressive changes, such especially as deposition of fat in the hyaline matter. The elastic fibrils are usually not increased and are sometimes reduced in number. Hydrostatic hypertensive and hypotensive influences are probably active in the causation of the splenic arterial and arteriolar hyalinosis.

The arteriolar hyalinosis found in cryptorchid testes is a second example of such localized hydrostatic reactions (Stroebe, Staemmler). The lumens of the arterioles of inguinally and abdominally retained testes are markedly narrowed and eccentric and are surrounded by thickened hyaline intima. The muscular media underneath in some of these vessels is atrophic and without nuclei and is replaced by hyaline matter. There is usually little lipid present. The internal elastic membrane is split. The youngest person in whom Staemmler observed such changes was 19 years old. Similar arteriolar lesions were not found in testes atrophic from myxedema, alcoholism, multiple sclerosis and renal arteriosclerosis. They are thus not the result of testicular atrophy. The large arteries of the testes are intact. While Staemmler suspected the action of a toxic factor in the development of the lesions, it is more likely that they are the result of a disproportion between the amount of blood supplied through the intact large testicular vessels and the capillary capacity of the organ remaining in its infantile state but receiving an amount of blood adequate for an adult, fully developed testis.

(c) Sclerosis of the Pulmonary Artery and Its Branches. The pulmonary artery, the anatomic structure of which resembles that of the aorta, differs from this vessel in its venous blood content and its low intravascular hydrostatic pressure, which is only about one third of that of the aorta (Hornowski). Meikel contended that this low pulmonary pressure is attributable to the extraordinary width of the lumens of the pulmonary arterioles, which are said to be four times as wide as those of the general circulation and which therefore offer a much lower peripheral resistance in the lesser circulation than is present in the systemic one. The pulmonary arterial pressure is moreover to a great extent independent of that in the general circulation and thus does not participate in most types of systemic hypertension (McCann, Katz and Stein-

itz) Further, vasoactive drugs affecting the aortic pressure usually leave the pulmonary pressure unchanged, while some drugs, such as beta-imidazolyethylamine, contract the pulmonary system but do not affect the vessels of the systemic circulation (Bredt). The blood pressure and content of the pulmonary vessels depend thus in general on the minute volume of the right ventricle, the resistance and capacity changes in the pulmonary vessels, and the "back-pressure resistance" developed in the left side of the heart by changes in the general circulation (Wiggers).

Deviations from this normal pattern causing an increase in the pulmonary intravascular hydrostatic pressure result from the following sources:

1. Elevation of the "back-pressure resistance" in the left side of the heart through failure of the general circulation, mitral stenosis or abnormal narrowness or other malformation of the pulmonary veins causing a reduction of the total cross surface of their orifices in the right auricle.

2. Delivery of increased amounts of blood into the pulmonary system because of shunting of arterial blood into the right chambers of the heart through a patent foramen ovale or through defects in the interauricular and interventricular septums, or into the pulmonary artery from the aorta through an open ductus arteriosus, or as a result of transposition of the large cardiac vessels.

3. Increase of the intrapulmonary circulatory resistance through reduction of the pulmonary arterial and capillary network, such as results from pneumoconiosis, chronic chemical pneumonia, chronic bronchitis and bronchiectasis, chronic fibrous pulmonary tuberculosis, pulmonary fibrosis associated with scleroderma, pulmonary schistosomiasis, emphysema, extensive pleural adhesions, pulmonary neoplasms, kyphoscoliosis or primary obliterating vascular disease of the lung representing either a part of periarteritis nodosa or of thromboangitis obliterans or appearing in response to the action of organ-specific vasospastic agents. A similar hemodynamic effect may be elicited in those parts of the pulmonary vessels which are not blocked by multiple emboli of the small pulmonary arteries and arterioles. Such emboli on the other hand, produce reduced intravascular hydrostatic pressure in the affected vessels. Stenosis of the stem of the pulmonary artery causes increased pressure only in the proximal part of this vessel and reduced pressure in the distal branches.

It is obvious from this analysis that atherosclerotic intimal thickenings of the stem of the pulmonary artery and its larger branches, which occur usually as a part of general atherosclerosis in older people and which are not uncommon, according to the observations of Brenner, do not furnish the basis for an increase of intravascular hydrostatic pressure in the pulmonary circulation but rather may cause a reduction, particularly if the atherosclerosis is complicated by mural thrombosis. It is also not likely that atheromatous reactions in the pulmonary artery are the

result of increased intrapulmonary pressure, as such vascular lesions, as will be shown later, are causally related to disturbances in the quantitative and qualitative physicochemical colloidal equilibrium of the lipid metabolism of the plasma. Such atheromatous lesions, however, may occur with and complicate the hydrostatic ones, especially in those cases in which arterial blood is shunted into the pulmonary artery (Hornowski, Brenner, Oguro).

Unrelated to the hydrostatic hypertensive changes in the pulmonary vessels are also those seen after rheumatic infections (Pappenheimer, Kugel, Chiarì) as well as those described by Wolff, which affect the media and resemble the cystic degenerative lesions noted in the ascending portion of the aorta by Erdheim, Gsell, Weise and many others. They must be distinguished, moreover, from the physiologic changes occurring in the pulmonary arteries with age (Brenner, Merkel). Inasmuch as the pulmonary vessels are highly distensible and thus can adapt themselves readily to considerable fluctuations in blood content (Parker), persistent and significant elevations of the intravascular hydrostatic pressure are not the result of minor interferences with, or increase of, the pulmonary flow of blood.

The figures for the incidence of pulmonary arterial sclerosis of all types vary greatly with different investigators, depending on the thoroughness of the postmortem examinations and on the use of gross or microscopic evidence. Whenever data based on macroscopic inspection are given, the values are distinctly lower than those based on microscopic studies, as these include also the changes in the small arteries and arterioles, which are of special importance in connection with the hydrostatic hypertensive type of pulmonary sclerosis. Bruning, who reviewed the early literature on this subject, stated that pulmonary angiosclerosis was considered rare during the last century by most workers (Otto, Andral, Albers, Dittrich, Bamberger, Cannstatt, Lobstein, Foerster, and many others). However, during this period there was no agreement among the various investigators as to the type of pulmonary sclerosis referred to, while some mentioned an endarteritic type of lesion, others noted atheromatous changes. Oppolzer, who first used the term "atheromatosis of the pulmonary artery," stated that this condition was relatively frequent.

This confusion extends even into more recent times. MacCallum recorded only 1 case of pulmonary arteriosclerosis of the primary endarteritic arteriolar type among 12,000 autopsies. Seely saw the condition once in 3,800 necropsies.



but observed that atheromatosis of the pulmonary artery was fairly common in persons with or without generalized atherosclerosis. The latter observation was confirmed by Ehlers. Miller stated that the rate of incidence of pulmonary arteriosclerosis of all types was 7.4 per cent, Moschowitz, 6.5 per cent, Costa, 8 per cent, Wartman, 6.4 to 8.6 per cent in all persons under 30 years of age and 40 to 58 per cent in persons over 60 years old, Ljungdahl, 50 per cent in persons over 50, all these data are based on gross examinations of the pulmonary arteries in routine autopsies. Microscopic studies of lungs and pulmonary arteries, on the other hand, have yielded much higher figures. Thus Costa found sclerotic lesions of the pulmonary artery in 65 per cent, while Brenner, who made a special study of this vascular disorder, was able to increase a macroscopic figure of 65 per cent to 97 per cent. Hueper furnished the only rate of incidence of pulmonary arteriosclerosis in animals available. He observed intimal hyalinosis and calcinosis in the large and medium-sized branches of the pulmonary artery in 15 per cent of white rats. It is noteworthy, however, that he did not find such or similar changes in routine histologic studies of the lungs of other experimental animals, such as mice, guinea pigs, rabbits, cats and dogs. While medial hyperplasia of the pulmonary arteries was not infrequent in rats, it was seen only occasionally in rabbits, cats and dogs.

The different parts of the pulmonary arterial tree participate in the sclerotic lesions, according to Brenner, in the following order. The stem is affected by patchy or diffuse, mainly fibrotic intimal thickenings in 56 per cent of the cases, large elastic branches show a similar involvement in 78 per cent of persons under 40 years and in 98 per cent of those over 40, small muscular arteries exhibit sclerosis in 90 per cent and arterioles in 94 per cent.

It is generally conceded that sclerosis of the pulmonary artery, especially that of hydrostatic genesis, occurs at an earlier age than that of the aorta. The average age was 32.7 years (range 16 to 49) in the series studied by Moschowitz, 28.6 years (range 9 to 49) in that of Ljungdahl and 31.9 years (range 13 to 58) in that of Miller. The youngest patient with pulmonary arteriosclerosis on record was a baby 6 months old, whose case was reported by Watjen. The age distribution of the 20 cases of so-called primary pulmonary arteriosclerosis in which a definite causal mechanism for the arteriosclerosis in the lung could not be demonstrated exhibits a

marked preponderance of cases in the younger age groups, according to Bill and Krygier

Years	0-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80
Cases	2	3	5	5	2	1	0	1

Males are apparently as often affected as females.

Among the various hydrostatic hypertensive mechanisms active in the production of pulmonary arteriosclerosis those of cardiovascular nature appear to be most important. Posselt noted that heart disease was present in the majority of 274 cases, and that in 40 per cent there was mitral stenosis. Brenner found cardiac disorders in 20 of 100 cases and general hypertension in 9 additional cases. Costa listed mitral stenosis in 18 cases, Miller in 15 cases. Steinberg in 29 cases, Parker and Weiss in 5 of 10 cases, Brunning in 5 of 21 cases, with 2 additional cases in which there were other mitral lesions and 1 in which there was obliterative pericarditis. Scheel recorded 4 cases with mitral stenosis and Oguro 3 cases. Steinberg observed pulmonary arteriosclerosis in 88 per cent of all cases of heart disease and in 83 per cent of 35 cases of heart disease with mitral stenosis. Miller, in 30 per cent of 52 cases of mitral stenosis, Zeek, in 59 cases among 62 cases of rheumatic heart disease. Additional cases of mitral stenosis with pulmonary arteriosclerosis were reported by Eliaschewitsch, Moschowitz, Lénart, Guilianni and others. Congestive induration of the lung was frequently accompanied by pulmonary arteriosclerosis (Bredt, Schutte). Congenital cardiac and aortic abnormalities figured in the following cases: open ductus arteriosus (Hohenstein, Johannsen and Connor, Zur Linden), patent foramen ovale and defects of the auricular or the ventricular septum (Watjen, Zur Linden, Romberg, Aust, Monckeberg, Johannsen and Connor, Posselt, Heuschen, Okkels and Theikelsen, Gombert, Stewart and Crawford), transposition of the aorta and the pulmonary artery (Watjen, Tonnes, Marchand). It may be mentioned in this connection that defects of the ventricular septum are not always congenital but may result from trauma to the chest (Husten, Revenstorf, Ebbinghaus, Fischer, Rosenthal, Gross, Terillou, Reubold). Thus any pulmonary arteriosclerosis developing on the basis of a traumatic defect of a septum has medicolegal significance. Hart noted narrowing of the orifices of the pulmonary veins as the cause of increased resistance in the pulmonary circulation.

The next frequent source of hydrostatic pulmonary hypertension is represented by processes reducing the vascular bed within the lung either by destruction of the capillaries as in emphysema

or by compression of these vessels as in pulmonary edema and pneumonia, or by interstitial fibrosing processes such as those seen in chronic pneumonia of physical or chemical genesis and in schistosomiasis, or by intravascular obliterating reactions such as endarteritic intimal proliferation, embolism and thrombosis, or by compression or collapse of pulmonary parenchyma as caused by tumors, extensive pleural adhesions and deformities of the chest, resulting in compensatory emphysema (Tugendreich, Sternbeig, Laubry and Parvu, Laubry and Thomas, Ayeiza, Rogeis, Warthin, Vaquez, Aruillaga, Aruillaga and Groux, Castex and Capdehouat, Schwartz, Munzer, Hohenner, Yater and Constant, Hoia, Konstam, Kuntschik, Rosenthal, Meyer, Mobitz, Kreiberg, and others)

Emphysema, which develops on an occupational basis in glass blowers and musicians playing brass instruments, was observed in 3 per cent of the 100 cases studied for pulmonary arteriosclerosis by Brenner, while pulmonary diseases of this and other types were present in 29 cases. Bruning recorded 5 cases of pulmonary disease, 2 of them with pleural obliteration, among a total of 21 cases of pulmonary arteriosclerosis. Fischer mentioned that pulmonary arteriosclerosis was always associated with pulmonary emphysema. Steinberg, on the other hand, saw this combination in only 82 per cent of his cases of emphysema, and Parker and Weiss did not see any sclerotic lesions in the pulmonary arteries in 15 cases of emphysema. Torhorst, again, listed cases of pulmonary arteriosclerosis associated with emphysema, as did a number of others (Brandes, Cook and Osborne, Miller, Moschowitz, Kaltreider). Parker studied 32 cases of emphysema and observed pulmonary arteriosclerosis in all except 6 (81.3 per cent), whereas Steinberg listed 50 per cent as the rate of incidence in his cases of emphysema. Parker mentioned that the degree of the arterial changes paralleled that of the emphysema. Torhorst recorded the occurrence of the pulmonary arterial lesions in connection with pulmonary tuberculosis and with pneumoconiosis, while others (Schlomka and Schulze, Hagemann, Wade, Seevers, Enzei and Becker, Miller, Linzbach and Wedler, Giese, Geilach, Gardner, Chair and Riddle, Bergerhoff, Gerstel, Kalbfleisch) saw pulmonary arterial and arteriolar sclerosis in cases of pulmonary silicosis and asbestosis. In this connection it is interesting to note that Gardner observed increased incidence of sclerosis of the general circulatory system with silicosis at age periods when silicosis is common. This observation may be related to the diminution in the oxygenation of the arterial blood that results

from the reduction of the total capacity of the silicotic lung with its decreased number of pulmonary vessels, decreased lumens and thickened alveolar walls. The latter change impairs the diffusing of oxygen through the alveolar walls, thereby eliciting hypoxemia, which is accentuated by the relatively increased deoxygenation of the blood in the tissues. Murphy, Kramin and Gerson, as well as Matsui, saw this condition in cases of generalized fibrosis of the internal organs, including the lung, complicating scleroderma.

The perivascular granulomatosis and fibrosis associated with the pulmonary schistosomiasis occurring endemically in South America, Egypt, Africa and Asia (Meira) and occasionally in North America (Cutler) was the cause of pulmonary arteriosclerosis in cases reported by Sorour, Bey, Miller and Clark and Graef.

Pulmonary arteriosclerosis in rats following production of pulmonary edema through prolonged inhalation of air with increased oxygen tension (80 per cent) has been reported (Smith, Bennett, Heim, Thomson and Drinker, Bennett and Smith, Smith and Bennett). This observation, however, was not confirmed in subsequent similar experiments of Rehbock, Oldt and Dixon. Darley and Doan considered the possibility that the chronic pulmonary edema elicited by excessive consumption of table salt (halophagia) might have been responsible for the development of pulmonary arteriosclerosis, and in view of the fact that in this case the repeated attacks of pneumonia caused several periods of increased pulmonary arterial pressure and may have been responsible for arteriolar endarteritis, it is more likely that a pneumonic mechanism of transitory hypertension accentuated and continued by secondary endarteritis was responsible for the ultimate pulmonary arteriosclerosis. It must be mentioned, however, that Kitamura attributed the pulmonary arteriosclerosis of his case to a general plethoric intravascular hydrostatic hypertension caused by habitual excessive consumption of beer.

Intrathoracic and pulmonary neoplasms compressing branches of the pulmonary artery and compressing or destroying a part of the vascular pulmonary bed were noted as causes of hydrostatic pulmonary hypertension and the ensuing pulmonary arteriosclerosis in some cases (Fried, Rossle, Hornowski, Lutensbacher), while thoracic deformities, such as kyphoscoliosis acting in similar fashion, figured in others (Edeiken, Ljungdahl).

Intravascular obstructions causing an elevation of the hydrostatic pressure in the rest of the pulmonary network have been observed. These took the form of repeated and multiple emboli in the

small pulmonary arteries in some instances (Ljungdahl, Krutzsch, Eppinger and Wagner, Lowenstein, Lang, Goedel) and the form of thromboangitic occlusions in these arteries in others (Lowenstein, Schutte, Wail, Bacon and Apfelbach). The hydrostatic mechanism in such cases differs fundamentally from that in some of the cases in which thrombi block partly or completely the stem or the main branches of the pulmonary artery, as in certain reported instances of pulmonary thrombosis (Desclin, Hart, von Jurgensen, Stadelmann, Monckeberg, Lowenstein, Ljungdahl, Goedel). While in several of these cases the thrombosis in the proximal parts of the vessel was secondary to similar changes in the distal portions and developed therefore on the basis of primary hydrostatic hypertension, Desclin noted in only 1 of his 6 cases of primary thrombosis of the pulmonary artery a minor degree of arteriosclerosis of the distal pulmonary vessels. Under such circumstances the hydrostatic pressure is increased only in the extrapulmonary part of the pulmonary artery, which is proximal to the thrombus, while in the intrapulmonary vessels, distal from it, hypotension prevails. Similar hemodynamic conditions are observed in cases of congenital stenosis of the pulmonary artery, usually affecting the stem at the region of the bifurcation. This malformation is rare as there are only 5 cases on record (Lissauer). The increased hydrostatic pressure in the proximal part of the pulmonary artery always dilates the lumen and in some cases causes the formation of an aneurysm. It may be mentioned in this connection that Karsner, Simon and Fujiwara, who injected suspensions of lycopodium seeds and of vanilla seeds in a solution of acacia daily for several months into the leg veins of dogs, some of which were exercised on a treadmill, failed to produce pulmonary arteriosclerotic changes by this method of eliciting hydrostatic hypertension in the lesser circulation.

There remain the 20 cases of so-called primary pulmonary arteriosclerosis (Monckeberg, Rosle, Sanders, Schutte, Ljungdahl, Hart, Krutzsch, Tschistowitsch, Goedel, MacCallum, Kuntschik, Ulrich, Brenner, Mallory, Killingsworth, Gibson) in which a primary cardiac or pulmonary mechanism could not be demonstrated and in which the action of some sort of primary peripheral pulmonary arterial spasm must be assumed, such as may be elicited by lead, nicotine and sympathicomimetic drugs (Brandes, Cook and Osborne, Lowenstein, Aust, Stadelmann, Bredt, Krutzsch). Such vasotonic agents in turn would be responsible for the endarteritic and thromboangitic reactions causing the per-

sistent pulmonary hydrostatic hypertension and the arterial and arteriolar sclerosis.

A dilatation of the stem and of the large branches of the pulmonary artery has been observed in several cases of pulmonary hydrostatic hypertension (Hart). Gombert noted that the small pulmonary arteries were distended and tortuous, Zur Linden, that the arterioles were dilated, forming sinusoid cavities. The lumens of the small and smallest arteries, however, were in most cases narrowed or obliterated through endarteritic fibrosing proliferations of the intima. In some cases, on the other hand, such partial or complete occlusions of the intrapulmonary arteries were produced by blood clots of thrombotic or embolic origin (Gombert, Parker and Weiss, Ljungdahl, Krutzsch, Goedel, Eppinger and Wail, Bacon and Apfelbach). These clots were often mural, situated at bifurcations and projected like polyps into the vascular lumens, suggesting thereby an embolic origin. They were in various stages of organization and recanalization and ultimately formed hemangiomatous structures (Gombert, Parker and Weiss, Ljungdahl, Krutzsch, Goedel, Eppinger and Wagner, Lowenstein) such as those seen in thromboangitis obliterans and in thrombophlebitis of the portal vein. In some of these cases as well as in others thrombotic processes in the small arteries and arterioles were associated with fibrinoid and necrotizing inflammatory changes in the vascular wall (Schutte, Wail, Bacon and Apfelbach). The necrotizing arteriolitis and arteritis thus produced resembled similar processes observed in thromboangitis obliterans (Benda), periarteritis nodosa (Jager) and malignant nephrosclerosis (Parker and Weiss).

Thrombosis of the stem and of the large branches of the pulmonary artery was seen in cases reported by Goedel and by Stewart and Crawford. The intima of these vessels exhibited thickenings of a fibrous and hyaline nature complicated by lipid deposits and calcifications. In the majority of cases of pulmonary arteriosclerosis either there were no intimal changes in these parts of the pulmonary vascular tree or there were white spots or stripes composed of fibrous and hyaline tissue (Brenner, Parker and Weiss). These lesions were most often located just above the cusps and in the region of the bifurcation. Similar changes of more extensive degree occurred in the medium-sized intrapulmonary arteries and were rarely complicated by lipid and calcific deposits. Degenerative fibrosing and hyaline changes in the media of the large and medium-sized pulmonary vessels occurred but were not common. Laubry, as well as Bredt,

stated that the sclerotic lesions were restricted to the large and medium-sized branches in cases of hydrostatic hypertension resulting from chronic pulmonary disease. However, there have been numerous cases of this genesis as well as of cardiac origin in which the intimal sclerosis extended to an increasing degree into the small and smallest arteries (Brenner).

The intimal changes observed in the small arteries and arterioles were in many cases those of oligocellular, fibrous proliferation of the endarteritic type. These reactions were particularly common in the cases of arteriosclerosis associated with pneumoconiosis, in which the blood flowing through the arterial channels of the lung is retarded and in which the parenchymatous fibrosis causes, through compression of arteries from the outside, localized hypotensive ischemic conditions in the pulmonary vascular tree. In a number of cases there were relatively cellular intimal proliferations in the arterioles. In others, especially those with congestive heart failure, there occurred subendothelial hyalinization and elastosis (Mobitz, Schutte, Kitamura, Rossle, Ljungdahl, Krutzsch, Steinberg, Bruning, Ehlers, Frey, Brenner). Muscular hypertrophy of the media was seen particularly often in cases of chronic pulmonary disease (Brenner). In some cases the arteriolar intimal thickenings contained appreciable numbers of lymphocytes (Schutte, Lang, Wagner and Eppinger, Murphy, Krainin and Gerson).

The arterial and arteriolar lesions occurring in the pulmonary arterial tree in association with increased intravascular hydrostatic pressure vary greatly in type and reflect the various and complex causal mechanisms and hemodynamic conditions complicating the main etiologic factor. It is for these reasons that the vascular reactions in hydrostatic hypertension of the lesser circulation resemble in many respects those found in the kidney with chronic glomerulonephritis or benign and malignant essential hypertension.

#### SUMMARY

The evidence presented as to the type of morphologic changes brought about in the arteries by an increase or a decrease of intravascular hydrostatic pressure shows that the lesions are very similar to those elicited by hypotensive and hypertensive conditions of vasotonic genesis. The main exception seems to be represented by the subendothelial hyalinosis and elastosis of the arterioles and small arteries seen only in connection with an increase of intravascular hydrostatic pressure.

#### INTRAVASCULAR HYDROSTATIC PRESSURE B INCREASED

- Adams, J. C. *J. Tennessee M. A.* **34** 423, 1941, *S. Clin. North America* **21** 1793, 1941.
- Afanassieff, W. A. *Beitr. z. path. Anat. u. z. allg. Path.* **8** 443, 1890.
- Arrillaga, F. C. *Monograph on cardiacos negros*, Buenos Aires, 1913, *Bull. et mem. Soc. med. de hôp. de Paris* **48** 292, 1924.
- Aschoff, L. *Verhandl. d. deutsch. path. Gesellsch.* **25** 106, 1930, *Klin. Wchnschr.* **18** 656, 1939.
- Aust, C. *München med. Wchnschr.* **39** 689, 1892.
- Bacon, C. M., and Apfelbach, C. W. *Arch. Path.* **3** 801, 1927, *Zentralbl. f. Path.* **40** 308, 1927.
- Baker, T. W., and Shelden, W. D. *Am. J. M. Sc.* **191** 626, 1936.
- Baucke, A. *Beitr. z. path. Anat. u. z. allg. Path.* **97** 307, 1936.
- Beckwith, J. R., and Chanutin, A. *Proc. Soc. Exper. Biol. & Med.* **46** 66, 1941.
- Beneke, R. *Frankfurt Ztschr. f. Path.* **28** 407, 1922, *Virchows Arch. f. path. Anat.* **287** 87, 1931.
- Benkowitz, K. B., and Hunter, W. C. *Am. J. Path.* **13** 289, 1937.
- Bennett, G. A., and Smith, F. J. C. *J. Exper. Med.* **59** 181, 1934.
- Bergerhoff, W. *Arch. f. Gewerbepath. u. Gewerbehyg.* **8** 339, 1937.
- Bey, S. A. *J. Egyptian M. A.* **15** 87, 1932.
- Brandes, W. W., Cook, R. A., and Osborne, M. P. *Arch. Path.* **36** 465, 1943.
- Bredt, H. *Virchows Arch. f. path. Anat.* **284** 126, 1932, *Klin. Wchnschr.* **10** 1930, 1931, **15** 1358, 1936, **18** 70, 1939.
- Brenner, O. *Arch. Int. Med.* **56** 211, 228, 457, 724, 976 and 1189, 1935, *Lancet* **1** 911, 1931.
- Brill, I. C., and Krygier, J. J. *Arch. Int. Med.* **68** 560, 1941.
- Brochner, R. J. *Arch. Path.* **28** 676, 1939.
- Bruning, H. *Beitr. z. path. Anat. u. z. allg. Path.* **30** 454, 1901.
- Buchner, F., and von Lucadou, W. *Beitr. z. path. Anat. u. z. allg. Path.* **93** 169, 1934.
- Cabot, R. C. *J. A. M. A.* **43** 774, 1904.
- Calh, G. *Arch. ital. di anat. e istol. pat.* **2** 835, 1931.
- Callander, C. L. *Johns Hopkins Hosp. Rep.* **19** 260, 1920, *Ann. Surg.* **71** 428, 1920.
- Castex, M. R., and Capdehourat, E. L. *Rev. Assoc. med. argent.* **57** 474, 1943.
- Charr, R., and Riddle, R. *Am. J. M. Sc.* **194** 502, 1937.
- and Savacool, J. W. *Arch. Path.* **30** 1159, 1940.
- Chiari, H. *Verhandl. d. deutsch. path. Gesellsch.* **9** 430, 1905.
- Clark, E., and Graef, I. *Am. J. Path.* **11** 693, 1935.
- Costa, A. *Clin. med. ital.* **59** 193, 1928, **58** 325, 1927.
- Cutler, M. *J. A. M. A.* **86** 816, 1926.
- Dandy, W. *Arch. Surg.* **17** 190, 1928.
- Darlev, W., and Doan, C. A. *Am. J. M. Sc.* **191** 633, 1936.
- Desclin, L. *Frankfurt Ztschr. f. Path.* **40** 160, 1930.
- Dill, L. V., and Isenhour, C. E. *Arch. Path.* **33** 655, 1942.
- Isenhour, C. E., Cadden, J. F., and Kuder, A. *Surg., Gynec. & Obst.* **72** 38, 1941.
- von Diringshofen, H. *Verhandl. d. deutsch. Gesellsch. f. Kreislaufforsch.* 1933, p. 146.
- and Belonoschkin, B. *Ztschr. f. Biol.* **93** 79, 1932.
- Eberhard, T. P. *Arch. Path.* **21** 616, 1936.
- Edeiken, J. *Am. J. M. Sc.* **186** 99, 1933.
- Ehlers, H. W. E. *Virchows Arch. f. path. Anat.* **178** 428, 1904.

- Ehaschewitsch, P A Virchows Arch f path Anat **279** 436, 1930
- Eppinger, H Die hepato-lienalen Erkrankungen (Pathologie der Wechselbeziehungen zwischen Milz, Leber und Knochenmark), Berlin, Julius Springer, 1920
- Ernst, P Beitr z path Anat u z allg Path **63** 141, 1916
- Escudero, P Arch d mal du cœur **19** 439, 1926
- Evans, F A Bull Johns Hopkins Hosp **24** 284, 1913
- Evans, W Quart J Med **2** 1, 1933
- Fahr, T Virchows Arch f path Anat **205** 397, 1911, Verhandl d deutsch path Gesellsch **13** 162, 1909, **15** 234, 1912
- Fischer, W Deutsches Arch f klin Med **97** 230, 1909
- Flaig, J Fortschr d Med **56** 89, 1938
- Froboese, C Beitr z path Anat u z allg Path **95** 496, 1935
- Gamm, K E J A M A **119** 134, 1942
- Gardner, L U M Clin North America **26** 1239, 1942
- Gerhartz, H Med Klin **20** 412, 1924
- Gerlach, W Arch f Gewerbepath u Gewerbehyg **2** 105 1931
- Gerstel, G Arch f Gewerbepath u Gewerbehyg **5** 249, 1934, **8** 277, 1937, Ueber die Veränderungen der Lungenblutgefäße bei Staublungenkranken, Jena, Gustav Fischer, 1933
- Giese, Klin Wchnschr **15** 731, 1936
- Goedel, A Virchows Arch f path Anat **277** 507, 1930
- Gorog, D Virchows Arch f path Anat **287** 602 1933
- Gombert, H Beitr z path Anat u z allg Path **91** 483, 1933
- Gordon, H, and Perla, D Am J Dis Child **41** 98, 1931
- Griffith, J Q, Jr Am J Physiol **122** 140, 1938
- Rutherford, R B, Roberts, E, and Lindauer, M A Am Heart J **21** 67, 1941
- Groedel, F M Exper Med & Surg **1** 94, 1943
- Guilianini, G B Pathologica **23** 321, 1931
- Guttmann, E Klin Wchnschr **6** 1808, 1927
- Hackel, W M Virchows Arch f path Anat **266** 630, 1928
- Harrison, F F Arch Path **27** 742, 1939
- Hart, C Berl klin Wchnschr **53** 304, 1916
- Harvey, H W Virchows Arch f path Anat **196** 303, 1909
- Hegemann, G Arch f Gewerbepath u Gewerbehyg **9** 228, 1938
- Heubner, W O Genussgifte in der Aetiologie der Herz- und Gefasskrankheiten (Alkohol, Kaffee, Nikotin) in Fortbildungslehrgang in Bad-Nauheim, 1937, vol 9, no 13, pp 24-26
- Heuschen, S E Samml klin Vortr, 1903-1907, no 105-136, p 595
- Hickl, W Frankfurt Ztschr f Path **41** 176, 1931
- Hohenner, K Arch f Kreislaufforsch **6** 293, 1940
- Holman, E Arteriovenous Aneurysm, New York, The Macmillan Company, 1937
- Hora, J Frankfurt Ztschr f Path **47** 100, 1934
- Hornowski, J Virchows Arch f path Anat **215** 280, 1914
- Hueper, W C Arch Path **20** 708, 1935
- Hultgren, J F J A M A **55** 279, 1910
- Husten, Verhandl d deutsch path Gesellsch **21** 249, 1926
- Irvine, A D Canad M A J **46** 436, 1942
- Israel, O Virchows Arch f path Anat **103** 461, 1886
- Ivy, A C J A M A **118** 569, 1942
- Jacob, F M J M Research **35** 187, 1916
- Johannsen, M W, and Connor, C A R Ann Int Med **18** 232, 1943
- Jongbloed, J, and Novons, A K Arch f d ges Physiol **233** 67, 1933
- Kalbfleisch, H H Arch f Gewerbepath u Gewerbehyg **4** 580, 1933
- Karsner, H T, Simon, M A, and Fujiwara, T F Arch Path **31** 585, 1941
- Katz, L N, and Steinitz, F S Am J Physiol **128** 433, 1940
- Kazda, F Mitt a d Grenzgeb d Med u Chir **38** 33, 1924
- Keys, A, and Butt, H R Arch Int Med **63** 165, 1939
- Klotz, O Centralbl f allg Path u path Anat **19** 535, 1908
- Koelsch and Lederer Arch f Gewerbepath u Gewerbehyg **1** 656, 1930
- Kohn, H Klin Wchnschr **8** 795 and 843, 1929
- Konstam, G L S Lancet **2** 756, 1929
- Krutzsch, G Frankfurt Ztschr f Path **23** 243, 1920
- Lake, M, Pratt, G H, and Wright, I S J A M A **119** 696, 1942
- Laubry, C, and Parvu, M Bull et mem Soc med d hop de Paris **27** 1320, 1909
- and Thomas, M ibid **50** 639, 1926
- Leary, T New England J Med **205** 231, 1931
- Lebert, H Berl klin Wchnschr **3** 60, 209, 229, 281, 336, 345, 386 and 402, 1866
- Lemon, W S Tr A Am Physicians **46** 340, 1931
- des Ligneris, M Heart **6** 249, 1916-1917
- Linden, W Virchows Arch f path Anat **252** 229, 1924
- Linzbach, A J, and Wedler, H W Virchows Arch f path Anat **307** 386, 1941
- Lissauer, M Virchows Arch f path Anat **180** 462, 1905
- Ljungdahl, M Untersuchungen über die Arteriosklerose des kleinen Kreislaufs, Wiesbaden, J F Bergmann, 1915
- Lowenstein, K Frankfurt Ztschr f Path **27** 226, 1922
- MacCallum, W G Bull Johns Hopkins Hosp **49** 37, 1931
- McCann, W S Arch Int Med **67** 680, 1941
- Mahrburg, S Virchows Arch f path Anat **274** 528, 1929
- Malkoff, G M Beitr z path Anat u z allg Path **25** 431, 1899
- Martland H S The Pathology of Acute and Chronic Alcoholism, in Emerson, H Alcohol and Man, New York, The Macmillan Company, 1935, p 201
- Matsuno, G Virchows Arch f path Anat **240** 69, 1922-1923
- Matthes, K, and Hochrein, M Arch f exper Path u Pharmakol **167** 687, 1932
- Meira, J A J A M A **117** 2090, 1941
- Merkel, H Beitr z path Anat u z allg Path **105** 176, 1941
- Miller, H R M J & Rec **136** 423 and 453, 1932
- M Clin North America **9** 673, 1925, Arch f Gewerbepath u Gewerbehyg **7** 126, 1936
- Miller, J L, and Williams, J L Am J M Sc **161** 327, 1921
- Miller, J W Verhandl d deutsch path Gesellsch **17** 265, 1914
- Moell, O H Beitr z path Anat u z allg Path **105** 366, 1941
- Monckeberg, J G Virchows Arch f path Anat **171** 141, 1903, Deutsche med Wchnschr **33** 1243, 1907

- Moschcowitz Virchows Arch f path Anat **283** 282, 1932, Am Heart J **6** 171, 1930, Am J M Sc **178** 244, 1929, **174** 388, 1927, Vascular Sclerosis, New York, Oxford University Press, 1942
- Muller, H Verhandl d deutsch path Gesellsch **19** 307, 1923
- Munzer, E Ergebn d ges Med **4** 162, 1923
- Murphy, J R, Krainin, P, and Gerson, M J J A M A **116** 499, 1941
- Nakonetschny Virchows Arch f path Anat **245** 564, 1923
- Nothnagel Ztschr f klin Med **15** 42, 1889
- Oguro, Y Virchows Arch f path Anat **198** 554 1909
- Okkels, H, and Therkelsen, F Acta path et microbiol Scandinav **9** 214, 1932
- Paine, C G, and Platt, R Brit M J **1** 698, 1931
- Parker, F, and Weiss, S Am J Path **12** 573, 1936
- Parker, L Ann Int Med **14** 795, 1940
- Pekelharing, C A Beitr z path Anat u z allg Path **8** 245, 1890
- Plesch, J Ztschr f klin Med **93** 241, 1922
- Poppen J R New England J Med **225** 892, 1941
- Posselt, A Ergebn d allg Path u path Anat **13** 298, 1909, Munchen med Wchnschr **55** 1625, 1908, Wien Arch f inn Med **11** 357, 1925
- Ranke, O F Luftfahrtmedizin **2** 243, 1938
- Read, W T, and Krumbhaar, E B M Clin North America **16** 237, 1932
- Rehbock, D J, Oldt M R, and Dixon, H M Arch Path **30** 1172, 1940
- Reid, M R Bull Johns Hopkins Hosp **31** 43, 1920 Am J Surg **14** 28 and 36, 1931
- Rossle, R Munchen med Wchnschr **55** 377, 1908
- Rogers, L Quart J Med **2** 1, 1908
- Romberg, E Deutsches Arch f klin Med **48** 197, 1891
- Rosenthal, S R Arch Path **10** 717, 1930
- Rowntree, L G J Pharmacol & Exper Therap **29** 135, 1926
- Ruffer, M A Studies on the Paleopathology of Egypt, Chicago, University of Chicago Press, 1921
- Saltykow, S Verhandl d deutsch path Gesellsch **14** 228, 1910
- Scheel, O Virchows Arch f path Anat **191** 135, 1908
- Schilling Verhandl d deutsch path Gesellsch **20** 154, 1925
- Schirokogoroff, J J Virchows Arch f path Anat **191** 482, 1908
- Schlomka, G, and Schulze, L Klin Wchnschr **13** 1208, 1934
- Schretzenmayr, A Arch f exper Path u Pharmacol **175** 284, 1934
- Schutte, H Zentralbl f Path **25** 483, 1914
- Seely, H J A M A **110** 792, 1938
- SeEVERS, M H, Enzer, N, and Becker, T J J Indust Hyg & Toxicol **20** 593, 1938
- Sella, H Beitr z path Anat u z allg Path **49** 501, 1910
- Smith, F J C, and Bennett, G A J Exper Med **59** 173, 1934
- Heim, J W, Thomson, R M, and Drinker, C K ibid **56** 63, 1932
- Bennett, G A, Heim, J W, Thomson, R M, and Drinker, C K ibid **56** 79, 1932
- Sorour, M F Proc Roy Soc Med **23** 1369, 1930
- Stadelmann Deutsche med Wchnschr **35** 1089 and 1122, 1909
- Staemmler, M Virchows Arch f path Anat **245** 304, 1923, Verhandl d deutsch path Gesellsch **19** 315, 1923, Zentralbl f allg Path u path Anat **34** 169, 1923-1924, Arch f Kreislaufforsch **3** 9, 1938
- Steinberg, U Beitr z path Anat u z allg Path **82** 307, 1929
- Steinmann, B Klin Wchnschr **17** 1640, 1938
- Stewart, H L, and Crawford, B L Am J Path **9** 637, 1933
- Stroebe Beitr z path Anat u z allg Path **22** 300, 1897
- Svdenstricker, E Statistical Study of Arteriosclerosis, in Cowdry, E V Arteriosclerosis, New York, The Macmillan Company, 1933, p 131
- Torhorst, H Beitr z path Anat u z allg Path **36** 210, 1904
- Tschistowitsch, T Compt rend Soc de biol **89** 627, 1923
- Tugendreich, J Ueber die Sklerose der Arteria pulmonalis, Inaug Dissert, Berlin, E Ebering, 1912
- Tuthill, C R Arch Path **16** 453, 1933, Arch Neurol & Psychiat **26** 268, 1931
- Vaquez, H Paris med **2** 15, 1926
- Voncken, J Frankfurt Ztschr f Path **42** 481, 1932
- Wade, J L West Virginia M J **36** 69, 1940
- Watjen Deutsche med Wchnschr **50** 713, 1924, Verhandl d deutsch path Gesellsch **21** 259, 1926
- Wallesch Virchows Arch f path Anat **251** 107, 1924
- Warthin, A S Tr A Am Physicians **34** 219, 1919
- Wartman, W B Am J M Sc **186** 27, 1933
- Weaver, J B Am J Surg **35** 586, 1937
- Westphalen, H Virchows Arch f path Anat **106** 420, 1886
- Wiggers Physiol Rev **1** 239, 1921
- Wolff, K Beitr z path Anat u z allg Path **35** 603, 1935
- Wright, A W Arch Path **32** 670, 1941
- Yater, W M, and Constam, G R M Clin North America **12** 1689, 1929
- Zeek, P Am J M Sc **184** 350, 1932
- Ziegler, E Verhandl d deutsch path Gesellsch **1** 85, 1898, Zentralbl f allg Path u path Anat **9** 844, 1898
- Zur Linden Virchows Arch f path Anat **252** 229, 1924

(To Be Continued)

## Notes and News

---

**Public Health Cancer Association**—This association is organized to meet the particular need of persons engaged in cancer control activities in federal, state, city and other official agencies. Active membership is limited to professional workers in official cancer programs. The president is Herbert L. Lombard, Boston, and the secretary-treasurer is Morton L. Levin, Albany, N. Y. A meeting and a symposium on cancer will be held in connection with the annual meeting of the American Public Health Association.

**Appointments**—Howard C. Hopps, of the University of Chicago, has been appointed professor of pathology and chairman of the department of pathology in the University of Oklahoma. He succeeds Louis A. Turley, who has retired as professor emeritus.

Sidney C. Madden, associate professor of pathology of the University of Rochester School of Medicine and Dentistry, Rochester, N. Y., has been appointed professor of pathology and head of the department of pathology at Emory University School of Medicine, Atlanta, Ga. The appointment fills the vacancy occasioned by Roy R. Kracke's acceptance of the deanship of University of Alabama School of Medicine.

**Deaths**—Sir John Ledingham, director of the Lister Institute of Preventive Medicine, London, England, died on October 4, 69 years of age. He served the institute for thirty-eight years, becoming director in 1931, after a long period as head of the department of bacteriology, serology and experimental pathology.

Alexis Carrel, of Paris, France, from 1912 to 1939 a member of the Rockefeller Institute for Medical Research and Nobel laureate, died on November 5 at the age of 71 years.

**Awards**—Roscoe R. Spencer, chief of the National Cancer Institute, has been given the Clement Cleveland Award for 1944 by the New York City Cancer Committee.

Through the American Academy of Pediatrics, Mead Johnson Awards have been presented to Fuller Albright, of Harvard Medical School, for his work on skeletal and metabolic disturbances in parathyroidism and other diseases affecting bone formation, and to Josef Warkany, of the Children's Hospital, Cincinnati, for his experimental study of congenital malformations induced by vitamin deficiencies in the mother's diet.

---

## Book Reviews

---

**Atlas of the Blood in Children**. By Kenneth D. Blackfan, M.D., late Thomas Morgan Rotch professor of pediatrics at Harvard Medical School and late physician in chief of the Infants' and Children's Hospital, Boston, and Louis K. Diamond, M.D., assistant professor of pediatrics at Harvard Medical School and visiting physician and hematologist of the Infants' and Children's Hospitals, Boston. With illustrations by C. Merrill Leister, M.D., associate pediatrician at St. Luke's Hospital, Bethlehem, and Allentown General Hospital, Allentown, Pa. Pp. 312, with 70 colored illustrations. Price \$12. New York: The Commonwealth Fund, 1944.

This atlas presents in painting and writing the results of systematic, comprehensive morphologic and clinical studies, over many years, of diseases of the blood in infancy and childhood. There are 70 plates of uniform excellence, with descriptive keys, illustrating blood cells in health and disease. The plates are accurate photolithographic reproductions of paintings by Dr. C. Mer-

rill Leister, pediatrician and artist. For the most part the originals were dry blood films stained with the Wright stain. The wish of the senior author, the late Dr. Blackfan, for good illustrations of the blood cells for the guidance of students and physicians has been well met indeed. The text consists of concise discussions of blood diseases with illustrative case histories, preceded by a description of the blood cells and a review of their origin. The maturation of the different series is illustrated in the first five plates. Then come sections on the erythrocytes in the various forms of anemia, on the leukocytes in disease, on leukemia and on the platelets. A short, select bibliography is appended. In the preface acknowledgment is made of the assistance and support of the Commonwealth Fund "which alone made possible the excellent reproduction of the plates and the publication of this atlas." It will be a most helpful guide to the study of blood diseases. A splendid addition has been made to the literature of clinical hematology.



## CONGENITAL GLYCOGENIC TUMORS OF THE HEART

THOMAS M. BATCHELOR, B.S., AND MARK E. MAUN, M.D.

DETROIT

Congenital tumors of the heart, rhabdomyomas, are comparatively rare. Since von Recklinghausen<sup>1</sup> described the first case in 1862, 63 authentic cases have been recorded. One atypical case was reported by Brown and Gray<sup>2</sup>. Four cases have been reported in other species, 1 in a guinea pig by Hueper,<sup>3</sup> 2 in swine by Hieronymi and Kukla<sup>4</sup> and Clausen,<sup>5</sup> and 1 in a bovine animal by Pires and Mucciolo.<sup>6</sup>

Knox and Schorer<sup>7</sup> were the first to record a case in American literature. Since that time cases have been reported by Wolbach,<sup>8</sup> Farber,<sup>9</sup> Yater,<sup>10</sup> Ill and Gray,<sup>11</sup> Wegman and Egbert,<sup>12</sup> Hueper,<sup>13</sup> Labate,<sup>14</sup> Olsen and Cooper<sup>15</sup> and Hillman.<sup>16</sup> The case reported here is the eleventh in American literature and the sixty-third in general medical literature.

### REPORT OF A CASE

The mother of the patient was a 19 year old primipara. She was admitted to the hospital Nov 24, 1943. The last menstrual period was Feb 25, 1943, the expected date of confinement, Dec 1, 1943. The prenatal and

the past history were noncontributory. The blood pressure was 180 systolic and 124 diastolic. The urine contained albumin (4 plus). The hemoglobin content was 10.5 Gm (66 per cent), the red cell count was 4,100,000, the white cell count, 11,000 with 72 per cent polymorphonuclear leukocytes. The Kahn test was negative.

On Nov 25, 1943 the mother spontaneously delivered an apparently normal full term boy, weighing 4 pounds 10¾ ounces (2,119 Gm). Respirations were slightly delayed, hence oxygen was given, with good results. Three days later the infant suddenly began to show intermittent cyanosis, which, however, soon became permanent and generalized. The heart sounds were normal. Loud moist rales were heard throughout both lungs. The infant pursued a rapidly downhill course and died November 28.

**Necropsy**—The autopsy was performed twenty hours after the patient died. The body was that of a white boy 53 cm in length and approximately 5 pounds (2,268 Gm) in weight. It was fairly well nourished, and development was good. Slight postmortem rigidity was present. Generalized patchy cyanosis of the skin was seen. No external anomalies were noted.

The bowel surfaces were smooth and shiny. About 50 cc of serous fluid was present in the peritoneal cavity. The liver weighed 65 Gm, was deep red and soft in consistency. Cut section revealed a marked excess of blood.

The lungs were free of adhesions. No fluid was present in the pleural cavities. Each lung weighed 20 Gm. Creptitation was diminished throughout, and the cut surfaces were moist and deep red.

The spleen weighed 8 Gm. Its cut surface revealed moderate congestion. Each kidney weighed 15 Gm. The organs were moderately soft in consistency, and their cut surfaces were deep red. No tumors or cysts were seen. The gastrointestinal tract showed no abnormalities. Unfortunately the brain was not examined.

The pericardial sac contained 5 cc of clear straw-colored fluid.

The heart measured 7.5 cm in width and was engorged with blood. When emptied, the heart with the roots of the great vessels weighed 32 Gm. Beneath the shiny visceral epicardium in the walls of the right and left ventricles were numerous raised, rounded tumor nodules, measuring 2 mm to 2 cm in diameter. Beneath the endocardium of the left ventricle there was a raised nodule, measuring 1.5 cm by 5 mm, that covered the interventricular septum. In the right ventricle two raised nodules, measuring 1 cm in diameter, were seen beneath the endocardium of the septum. The papillary muscles in both ventricles showed small nodules, about 2 mm in diameter. Similar nodules were seen in both auricles.

On cut section the nodules were fairly well defined but nonencapsulated, they were subepicardial, intramural and subendocardial in location. Their cut sur-

From the Department of Pathology of Wayne University College of Medicine and St Mary's Hospital.

1 von Recklinghausen. *Monatschr f Geburtsch u Frauenkr* 20:1, 1862.

2 Brown, G., and Gray, J. *Lancet* 1:915, 1930.

3 Hueper, W. C. *Am J Path* 17:121, 1941.

4 Hieronymi, E., and Kukla, R. *Virchows Arch f path Anat* 232:459, 1921.

5 Clausen, L. *Deutsche tierarztl Wchnschr* 46:838, 1938.

6 Pires, E. R., and Mucciolo, P. *Rev Fac Med Vet Univ São Paulo* 1:67, 1939.

7 Knox, J. H. M., and Schorer, E. H. *Arch Pediat* 23:561, 1906.

8 Wolbach, S. B. *J M Research* 16:495, 1907.

9 Farber, S. *Am J Path* 7:105, 1931.

10 Yater, W. M. *Arch Int Med* 48:627, 1931.

11 Ill, C. H., and Gray, F. W. *Am J Obst & Gynec* 28:264, 1934.

12 Wegman, M. E., and Egbert, D. S. *J Pediat* 6:818, 1935.

13 Hueper, W. C. *Arch Path* 19:372, 1935.

14 Labate, J. S. *Am J Path* 15:137, 1939.

15 Olsen, R., and Cooper, R. J. *Am J Path* 17:125, 1941.

16 Hillman, R. W. *Brooklyn Hosp J* 3:181, 1941.



faces were yellowish white, smooth and somewhat softer in consistency than the adjacent brownish red myocardium. The valve leaflets were normal in appearance. A patent foramen ovale, measuring 1 cm in diameter, was present, and a ductus arteriosus, measuring 3 mm in diameter, connected the pulmonary artery with the aorta.

The heart was immediately placed in a 4 per cent solution of formaldehyde, but it was removed two hours later, and blocks of tissue were placed in absolute alcohol, in Zenker's solution and in a 4 per cent solution of for-

maldehyde-fixed tissue from the heart were stained with sudan III.

*Microscopic Examination*—Sections of the nodules stained with hematoxylin-eosin presented the typical vacuolated appearance described in the literature. The nodules were 500 microns to 2 cm in diameter. They appeared discrete because of the numerous clear spaces within the cells of the tumors that were in contrast to the more compact eosin-staining cytoplasm of the surrounding myocardial fibers. The boundaries of the nodules for the most part were sharp,

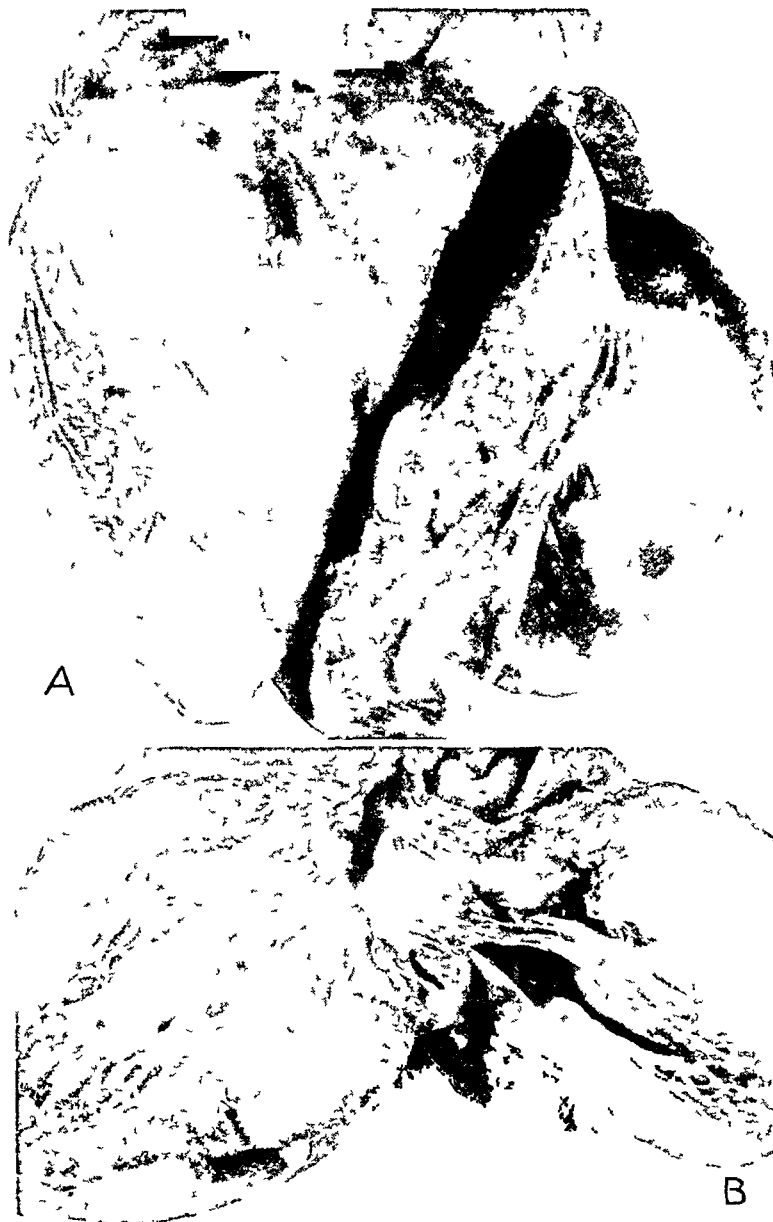


Fig 1—*A*, heart with raised tumor nodules of varying sizes protruding from beneath the epicardium of the right ventricle. Nodules may be seen within the myocardium of the open portions of the heart. *B*, left ventricle opened to show raised subendocardial nodule located in the interventricular septum. Other tumors are seen within the myocardium.

maldehyde. Blocks of all the tissues were embedded in paraffin and stained with hematoxylin-eosin. In addition, prepared sections of the heart were stained with azocarmine and with phosphotungstic acid-hematoxylin. Other sections of the heart that were fixed in absolute alcohol and embedded in celloidin (a concentrated preparation of pyroxylin) were cut and stained with Best's carmine stain for glycogen. Frozen sections of formal-

but in some areas a gradual transition from normal cardiac fibers to the vacuolated cells was apparent. A few blood vessels were seen within the tumors. Sections of the tumors stained with hematoxylin-eosin revealed under higher magnification that the nodules were composed of closely packed cells ranging from 8 to 300 microns in diameter. The cells contained large empty spaces with only a small

peripheral rim of eosin-staining cytoplasm. The nuclei when present were located chiefly in the peripheral strands of cytoplasm. Occasionally, however, they were located centrally in the vacuolated cells, surrounded by a narrow zone of cytoplasm that extended to the periphery in spider web fashion.

Sections of the myocardium stained by Best's carmine method revealed numerous glycogen granules within the vacuolated spaces of the tumor cells. Some cardiac fibers that appeared normal with routine hematoxylin-eosin stains were also found to contain fine granules of glycogen.

Sections of the myocardium stained with azocarmine revealed a small amount of connective tissue within the

tions. From so striking a picture it is easy for one to believe that the granular appearance of the compressed cytoplasm of the tumor cells represents no more than the cut ends of cross striations. No mitotic figures were seen.

When stained with sudan III, sections of the tumors revealed a few fat droplets within the sarcoplasm of the cells rather than within the vacuolated spaces themselves.

Histologic examination of the other organs revealed bronchopneumonia and partial atelectasis of each lung and acute passive congestion of the liver, the spleen and the kidneys. No glycogenic tumor nodules were found in the other organs and tissues examined.

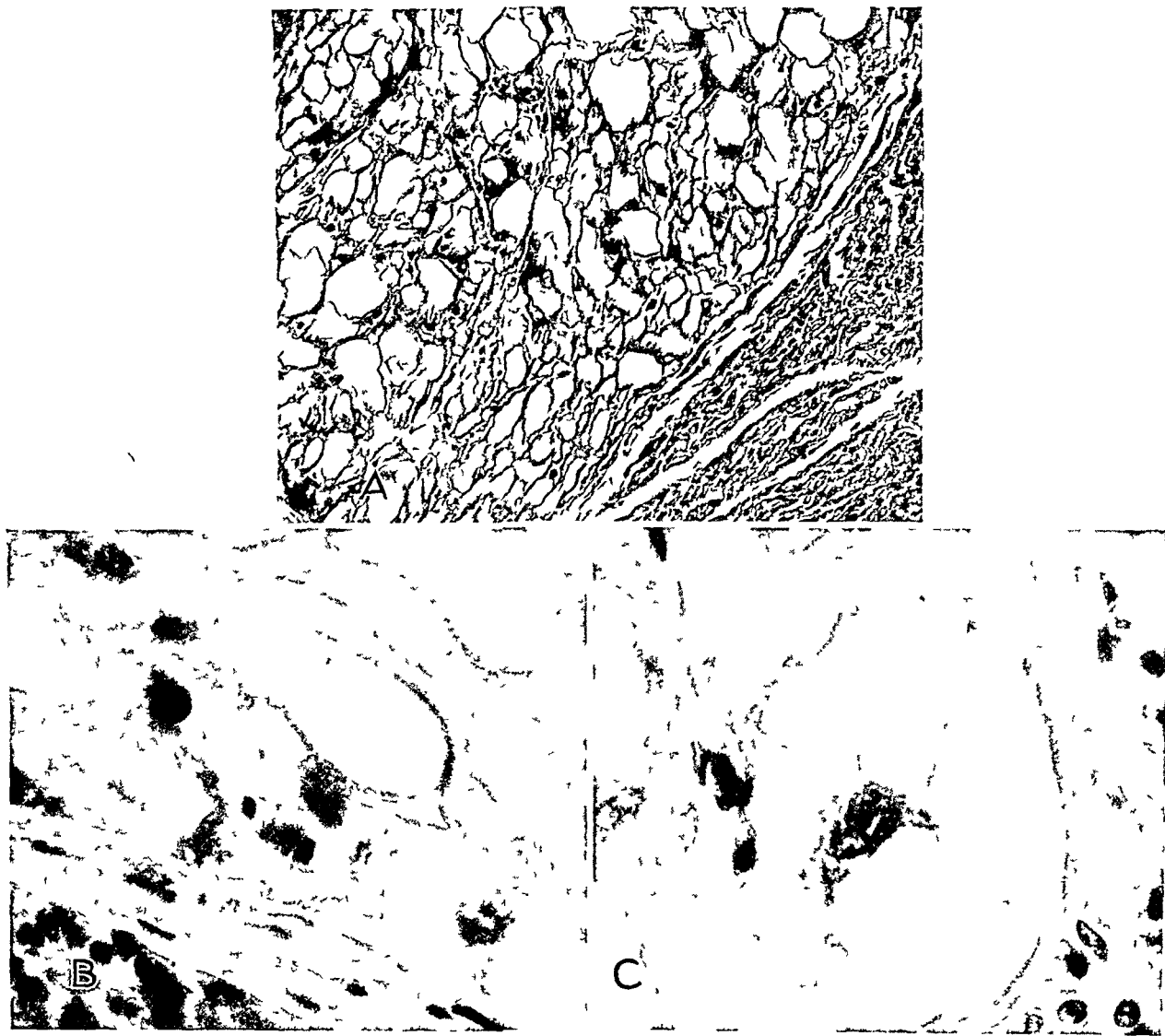


Fig 2—*A*, photomicrograph of a nodule and the adjacent normal myocardium. Note marked vacuolation of the tumor cells. Hematoxylin-eosin,  $\times 100$ . *B*, higher magnification of the edge of the nodule. Note the apparent sharp transition from normal cardiac fibers to vacuolated cells. Hematoxylin-eosin,  $\times 600$ . *C*, photomicrograph showing a typical spider cell. Note the central location of the nucleus and the delicate cytoplasmic strands having a spider web arrangement. Hematoxylin-eosin,  $\times 600$ .

vacuolated tumors and in association with the blood vessels. The normal cardiac fibers stained red, and cross striations within the cytoplasm of the fibers were visible. The vacuolated cells of the tumors were completely surrounded by red-staining granular cytoplasm.

With the aid of phosphotungstic acid-hematoxylin stain, the cytoplasmic granules resembled myofibrillar material. Further, in some areas horizontal sections of the peripheral septums of the vacuolated cells were seen as thin sheaths of sarcoplasm with distinct cross stri-

#### COMMENT AND REVIEW OF THE LITERATURE

*Terminology*—There has been considerable disagreement over the name that should be attached to the entity under discussion. "Rhabdomyoma" was used by early investigators and has been the term generally accepted by contemporary students of the subject even though they agree that the tumors to which it is attached do not

represent a neoplastic process Wolbach,<sup>8</sup> using phosphotungstic acid-hematoxylin stain, demonstrated early fibril formation of the cardiac muscle and with this evidence considered the rhabdomyoma a true neoplasm. On the other hand Steinbiss,<sup>17</sup> Olsen and Cooper,<sup>15</sup> Yate<sup>10</sup> and Hueper<sup>13</sup> endorsed the term "hamartoma" as used by Albrecht,<sup>18</sup> to indicate a tumor-like, nodular, congenital lesion that did not represent a proliferative neoplastic lesion. Olsen and Cooper<sup>15</sup> have offered the term "congenital nodular glycogenic degeneration of the myocardium" to indicate a localized degenerative process involving the myocardium of the newborn. These authors expressed the belief, along with the others, that "congenital rhabdomyomatosis" represented a degenerative lesion because of the presence of glycogen in the vacuolated cells of the tumors. Steinbiss<sup>17</sup> expressed the belief that fibrosis and calcification in older lesions represented degenerative phenomena. Humphreys and Kato<sup>19</sup> suggested that the diffuse type of "rhabdomyomatosis" may be a form of Gierke's storage disease. Hueper,<sup>3</sup> on the other hand expressed the belief that the glycogen present in the nodular tumors of the heart differed from that found in Gierke's storage disease because in the former the glycogen dissolved out readily in ordinary tissue fixatives while in the latter the glycogen could be demonstrated in the cells weeks to months later. To summarize, "rhabdomyoma" has become firmly fixed in the literature, but it is a misnomer in that no true neoplasm occurs. "Congenital nodular glycogenic degeneration of the myocardium" is not accurate because there is no evidence to support the contention that accumulations of glycogen within myocardial fibers represent degenerative phenomena, neither do fibrosis and calcification in older lesions represent degeneration. "Hamartoma" as used by Albrecht<sup>18</sup> is a more suitable term in that it indicates the congenital nature and the lack of neoplastic qualities of the nodular lesions, but it does not emphasize the most important feature of the entity, the glycogen content.

*Origin*—The origin of the nodular glycogenic tumors has been the subject of considerable speculation. Knox and Schorer,<sup>7</sup> Kawamura,<sup>20</sup> Schulgin,<sup>21</sup> Monckeberg,<sup>22</sup> Abricosoff,<sup>23</sup> Uehlin-

ger<sup>24</sup> and Berger and Vallee<sup>25</sup> expressed the belief that the tumor cells arose from the Purkinje system. However, since lesions could be found in areas where Purkinje fibers were not located, other investigators doubted this theory. Bundschuh<sup>26</sup> and Amersbach and Handorn<sup>27</sup> showed the constituent fibers to be of extra-Purkinje origin. Many observers noted the similarity between the tumor cells and embryonic myocardial fibers. Bundschuh<sup>26</sup> demonstrated that the tumors were composed of a more primitive muscle tissue that was present before differentiation into the two adult constituents of the myocardium occurred. According to Bonome-Cagnetto,<sup>28</sup> degenerated cardiac fibers were replaced in fetal life by connective tissue whose overgrowth caused the isolation of islands of embryonic heart muscle fibers, these fibers gave rise to the tumor cells.

*Clinical Symptoms*—Glycogen tumors of the heart rarely cause clinical symptoms except in those patients in whom the tumors are situated on the valve leaflets, in the event of this location murmurs may be heard. Wegman and Egbert<sup>12</sup> reported a case in which cardiac arrhythmia was detected clinically, they concluded that the disorder was due to involvement of the conducting system. Cyanosis was a frequent symptom in the cases reviewed, especially in newborn infants and in those patients who died suddenly.

Since tuberous sclerosis was associated with congenital nodular cardiac tumors in a large number of cases, one would expect symptoms of a neurologic nature, such appeared. In children and infants, mental development was retarded, and the ability to walk and talk was delayed, some exhibited marked indifference to their surroundings. In some of the patients epileptiform attacks were frequent. However, one of the most constant findings was the occurrence of sudden death after minor ailments from which recovery was usually expected.

*Incidence*—So-called congenital rhabdomyoma occurs chiefly in the newborn and infants under 1 year of age. In the series studied, totaling 63

22 Monckeberg, J. G. *Munchen med Wchnschr* **61** 2108, 1914

23 Abricosoff, A. J. *Beitr z path Anat u z allg Path* **45** 376, 1909

24 Uehlinger, E. *Virchows Arch f path Anat* **258** 719, 1925

25 Berger, L., and Vallee, A. *Ann d'anat path* **7** 797, 1930

26 Bundschuh, E. *Beitr z path Anat u z allg Path* **54** 278, 1912

27 Amersbach and Handorn. *Frankfurt Ztschr f Path* **25** 124, 1921

28 Bonome-Cagnetto, A. *Atti d r Ist Veneto di sc, lett e arti* **5** 205, 1903

17 Steinbiss, W. *Virchows Arch f path Anat* **243** 22, 1923

18 Albrecht, E. *Verhandl d deutsch path Gesellsch* **8** 89, 1904

19 Humphreys, E., and Kato, K. *Am J Path* **10** 589, 1934

20 Kawamura, R. *Centralbl f allg Path u path Anat* **24** 801, 1913

21 Schulgin, M. *Zentralbl f Herz- u Gefässkr* **5** 33, 1913

cases, the incidence in the various age groups was as follows

Newborn	15
Under 1 year	18
1 to 3 years	9
3 to 15 years	12
Over 15 years	7
Not given	2
Total	63 cases

The disease affects both males and females but is more common in the former. As for racial distribution, it has been reported in Negroes (Hueper<sup>13</sup> and Yater<sup>10</sup>) and in Japanese (Tamura<sup>29</sup> and Mitani<sup>29a</sup>) as well as in the white race.

*Description of Tumors*—Glycogenic tumors involve the left ventricle more frequently than any other site, however, any portion of the heart, including the valve leaflets, may be involved by nodules. The tumors may be subepicardial, intramural or subendocardial. In so-called congenital rhabdomyomatosis one may see any of three pictures. In 11 of the cases reviewed a single tumor was found within the myocardium, usually in the region of the apex of the heart, in 48 of the cases multiple nodules involved almost every portion of the heart, and in 3 cases there was diffuse involvement of the myocardium, the case reported by Schmincke<sup>30</sup> being the most remarkable in that the heart was hypertrophied and the entire myocardium replaced by rhabdomyoma fibers while the normal contour of the heart was retained.

When nodules are seen within the myocardium, they usually appear as discrete, homogeneous, yellowish white areas, somewhat soft in consistency. However, in the case reported by Brown and Gray<sup>2</sup> a single tumor nodule, measuring 3 by 2¼ inches (7.5 to 5.6 cm.) and found in the lateral wall of the left ventricle, presented a pale white cut surface and a distinct coarse fasciculated appearance closely resembling that of a fibroma. Microscopically, the tumor was composed of interlacing bundles of cardiac muscle fibers separated by the abundant connective tissue that formed the bulk of the tumor. Only a few microscopic areas showed slight vacuolation. We feel that this case should not be considered in the same category as the others discussed in this paper because of the absence of typical features described in the cases in the literature and noted in the case we have reported.

Earlier writers were not able to identify definitely the contents of the vacuolated areas or to

appreciate their significance. Von Recklinghausen<sup>1</sup> considered the empty spaces to represent lymph or blood channels or "muscle-tubes" of pathologic origin. Virchow<sup>31</sup> thought they were either lymphatic cavities or clear serous spaces. Hlava<sup>32</sup> considered them to be artefacts of fixation. Of the early writers, Ponfick,<sup>33</sup> Knox and Schorer<sup>7</sup> and Wolbach<sup>8</sup> considered them to be intracellular spaces. In regard to the content of the vacuolated spaces Marchand<sup>34</sup> suggested that glycogen might be present because of the similarity of the tumor cells to fetal myocardium. Seiffert<sup>35</sup> was convinced that glycogen was present, although he was unable to demonstrate it clearly. Monckeberg<sup>22</sup> and Rehder<sup>36</sup> demonstrated glycogen in the tumor cells, and other investigators since then have obtained stains proving that glycogen was present within the vacuoles. Bundschuh<sup>26</sup> found fine droplets of fat in cardiac fibers near the periphery of the vacuolated nodules. Kawamura,<sup>20</sup> Mittasch,<sup>37</sup> Olsen and Cooper<sup>15</sup> and Rae<sup>38</sup> demonstrated fine fat droplets in the cytoplasm of the cardiac fibers. Steinbiss<sup>17</sup> stated definitely that the vacuoles were entirely free from fat, a fact that has been substantiated by later investigators.

Cesaris-Demel<sup>39</sup> was the first to mention the so-called "spider cells" as many-processed cells lying within the vacuolated areas, he considered the spaces to be intercellular. Seiffert<sup>35</sup> compared these "spider cells" to huge embryonic cells. After careful study of the present case, we are convinced that the "spider cell" does not represent a cell within a vacuolated space, it merely represents a striated muscle cell that has become vacuolated by intracellular accumulations of glycogen in such a manner that the nucleus of the cell remains centrally located, surrounded by a narrow zone of cytoplasm, while the remaining cytoplasm assumes a spider web pattern.

*Associated Lesions*—Congenital glycogenic tumors of the heart usually occur in association with some other lesions. Von Recklinghausen<sup>1</sup> was the first to note the presence of tuberous sclerosis in cases of rhabdomyoma of the heart.

31 Virchow, R. Virchows Arch f path Anat **30** 468, 1864

32 Hlava, J. Prazd **1** 376, 1887

33 Ponfick. Verhandl d deutsch path Gesellsch **4** 226, 1901

34 Marchand. Verhandl d deutsch path Gesellsch **3** 64, 1901

35 Seiffert. Verhandl d deutsch path Gesellsch **3** 64, 1901

36 Rehder, H. Virchows Arch f path Anat **217** 174 1914

37 Mittasch. München med Wchnschr **69** 571, 1922

38 Rae, M. V. Canad M A J **39** 63, 1938

39 Cesaris-Demel, A. Arch per le sc med **19** 139, 1895

29 Tamura, O. Gann **30** 391, 1936

29a. Mitani, S. Tr Soc path jap **24** 589, 1934

30 Schmincke, A. Beitr z path Anat u z allg Path **70** 513, 1922

	Author	Heart	Glycogen	Brain	Other Organs	Age
1	Von Recklinghausen <sup>1</sup>	Multiple nodules		Tuberous sclerosis	Cutaneous tumor	Newborn
2	Virchow <sup>31</sup>	Multiple nodules			Large liver, tumors of skin	Newborn
3	Hlava <sup>32</sup>	Single nodule				14 days
4	Kolisko, A. Med Jahrb 2 135 1887	Multiple nodules				2 months
5	Cesaris Demel <sup>30</sup>	Multiple nodules		Tuberous sclerosis	Multiple renal adenoma	3 years
6	Seiffert <sup>30</sup>	Multiple nodules			Cysts of kidney	20 months (M)
7	Seiffert <sup>30</sup>	Multiple nodules				7 months
8	Rothe Allg med Centr Ztg 70 175, 1901	Multiple nodules		Tuberous sclerosis	Tumors of breast	
9	Ponfick <sup>33</sup>	Multiple nodules		Tuberous sclerosis		7 months
10	Ponfick <sup>33</sup>	Multiple nodules		Tuberous sclerosis		3 years (M)
11	Bonome Cagnetto <sup>28</sup>	Multiple nodules		Tuberous sclerosis		18 months (F)
12	Riedmatten, R Trav d l'Inst path d Lausanne 3 167, 1904	Multiple nodules		Tuberous sclerosis		7 months
13	Knox and Schorer <sup>7*</sup>	Multiple tumors			Multiple neuroglioma	7 months
14	Wolbach <sup>8*</sup>	Single tumor				10 months (F)
15	Abricoff <sup>23</sup>	Multiple nodules	Present	Tuberous sclerosis		3½ years
16	Ehrnrooth, E Beitr z path Anat u z allg Path 51 262, 1911	Single tumor				7 months
17	Bundschuh <sup>26</sup>	Multiple nodules		Tuberous sclerosis	Renal tumors and cysts, tumors of skin	2 years (F)
18	Jonas W Frankfurt Ztschr f Path 11 105, 1912	Multiple nodules		Tuberous sclerosis	Harelip, cleft palate, anomalous kidney	6 months (M)
19	Kawamura <sup>20</sup>	Multiple nodules			Renal tumors, congenital anomalies of pancreas, esophagus, rectum	4 years (F)
20	Schulgin <sup>21</sup>	Multiple nodules		Tuberous sclerosis	Renal tumors	6 days
21	Schulgin <sup>21</sup>	Multiple nodules		Tuberous sclerosis		6 years
22	Rehder <sup>36</sup>	Multiple nodules	Present			Newborn
23	Monckeberg <sup>22</sup>	Multiple nodules	Present		Absence of right kidney and ureter	14 months
24	Ribbert, H Centralbl f allg Path u path Anat 26 241, 1915	Multiple nodules		Tuberous sclerosis	Renal cysts	1 year
25	Hisinger Jagerskiold, E Finska lak sallsk Handl 58 953, 1916	Single tumor			Renal tumors	7½ months
26	Melnikov Razvedenkov <sup>40</sup>	Single tumor				20 25 years (M)
27	Amersbach and Handorn <sup>27</sup>	Single tumor				7 days (M)
28	Kaufmann, E Lehrbuch der speziellen pathologischen Anatomie fur Studierende und Aerzte, Berlin W de Gruyter & Co, 1922	Multiple nodules		Tuberous sclerosis		3 years
29	Kaufmann, E Ibid	Multiple nodules		Tuberous sclerosis	Renal tumors	7 years
30	Mittasch <sup>37</sup>	Multiple nodules		Tuberous sclerosis	Renal tumors	4 months
31	Mittasch <sup>37</sup>	Multiple nodules		Tuberous sclerosis	Renal tumors	14 years (M)
32	Mittasch <sup>37</sup>	Multiple nodules		Tuberous sclerosis	Renal tumors, angiomyolipoma of liver	31 years (M)
33	Schmincke <sup>30</sup>	Diffuse involvement			Congenital tumors of lungs	Newborn
34	Steinbiss <sup>17</sup>	Multiple nodules		Tuberous sclerosis	Fibroepithelioma of skin	5 years (M)
35	Steinbiss <sup>17</sup>	Multiple nodules		Tuberous sclerosis	Renal tumors	3 years (M)
36	Steinbiss <sup>17</sup>	Multiple nodules		Tuberous sclerosis	Renal and cutaneous tumors	10 years (M)
37	Steinbiss <sup>17</sup>	Single tumor with cicatrix		Tuberous sclerosis	Renal tumors and cysts	16 years (M)
38	Steinbiss <sup>17</sup>	Multiple nodules		Tuberous sclerosis	Renal tumors	21 years (F)
39	Steinbiss <sup>17</sup>	Single tumor		Tuberous sclerosis	Renal tumors	35 years
40	Omodei Zorini, A Arch per le sc med 46 97, 1923	Single tumor				2½ years
41	Uehlinger <sup>24</sup>	Multiple nodules				20 years
42	Sikl <sup>41</sup>	Single nodule				9 weeks (M)
43	Berger and Vallee <sup>20</sup>	Multiple nodules			Renal cysts	2 years
44	Farber <sup>9*</sup>	Multiple nodules	Present	Tuberous sclerosis	Renal cysts	6 months (F)
45	Yater <sup>10*</sup>	Multiple nodules		Tuberous sclerosis	Renal tumor	5 years (F)
46	Reitano, R, and Nucciotti, L Cuore e circolaz 17 605, 1933	Multiple nodules				1 day
47	Baruchev <sup>42</sup>	Single nodule				3 days
48	Ill and Gray <sup>11*</sup>	Multiple nodules			Hepatomegaly and splenomegaly	48 hours (M)
49	Lamburner <sup>43</sup>	Multiple nodules				45 years (F)
50	Mitani <sup>29a</sup>	Multiple nodules		Tuberous sclerosis	Renal cysts	Newborn (M)
51	Wegman and Egbert <sup>12*</sup>	Multiple nodules			Renal cysts	10 months (F)
52	Hueper <sup>13*</sup>	Multiple foci (microscopic)	Present	Tuberous sclerosis	Renal cysts, hepatomegaly, multiple spongioblastoma of basal ganglions	7 months (M)
53	Pauli, W Monatschr f Kinderh 66 22 1936	Diffuse tumor			Hepatomegaly, hydrocele	4 months (M)
54	Pauli W Ibid	Diffuse tumor				6½ months (M)
55	Tamura <sup>29</sup>	Multiple nodules				
56	Elhak <sup>45</sup>	Multiple nodules			Polycystic kidneys	24 hours (F)
57	O'Flynn and Mackay <sup>44</sup>	Multiple nodules		Tuberous sclerosis		8½ months (M)
58	Rae <sup>38</sup>	Multiple nodules			Hepatomegaly, splenomegaly, partial atelectasis of lungs	3 days (M)
59	Stewart <sup>46</sup>	Multiple nodules		Tuberous sclerosis	Renal cysts	9 months (M)
60	Labate <sup>11*</sup>	Multiple nodules		Tuberous sclerosis	Anomaly of heart	3 hours (M)
61	Hillman <sup>16*</sup>	Multiple nodules	Present	Tuberous sclerosis		21 months (F)
62	Olsen and Cooper <sup>10*</sup>	Multiple nodules	Present		Hypertrophic pyloric stenosis 4 lobes to right lung, atelectasis of both lungs, renal abscess	40 days (M)
63	Batchelor T M and Maun M E Arch Path, this issue *	Multiple nodules	Present		Partial atelectasis of both lungs	3 days (M)

\* Cases reported in American literature

but he failed to describe the lesion. Likewise Cesariis-Demel<sup>39</sup> failed to describe this condition that occurred in association with rhabdomyoma of the heart in the case which he reported. It remained for Ponfick<sup>3</sup> to give a clear description of the associated tuberous sclerosis in his 2 cases of rhabdomyoma. He sought to explain both on a common basis of fetal malnutrition with secondary vascular changes. The tumors in all 6 cases reported by Steinbiss<sup>17</sup> were associated with tuberous sclerosis. In a review of the literature he found that lesions of the brain occurred in 1 of 4 cases in which the heart was involved by a single nodule, in contrast to 24 of 28 cases in which the heart was involved by multiple tumors. Labate,<sup>14</sup> in a comprehensive review of the literature, reported tuberous sclerosis in 29 of 51 cases reviewed. However, in cases not included in the review, namely, those of Melnikov-Razvedenkov,<sup>40</sup> Sikl,<sup>41</sup> Yater,<sup>10</sup> Baruchev,<sup>42</sup> Lymburner,<sup>43</sup> O'Flynn and Mackay,<sup>44</sup> Eliakis,<sup>45</sup> Rae<sup>38</sup> and Stewart,<sup>46</sup> tuberous sclerosis was reported by Yater,<sup>10</sup> O'Flynn and Mackay<sup>44</sup> and Stewart<sup>46</sup>. Since 1939, 2 additional cases, exclusive of the present case, have been added to the literature. Hillman<sup>16</sup> reported tuberous sclerosis

The brain was not examined by Olsen and Cooper<sup>15</sup>.

Although the present review reveals that tuberous sclerosis has been observed in 50 per cent of the cases of congenital nodular glycogenic tumors of the heart, there is no doubt that the incidence of coexistence of the two lesions is much higher. Other congenital disturbances that are frequently seen with these tumors include cysts and tumors of the kidneys, tumors of the sebaceous glands, harelip, cleft palate, multiple glioma of the brain and congenital malformations of the pancreas, the kidneys and the lungs.

#### SUMMARY

In a case of multiple tumors of the heart in a 3 day old boy glycogen was demonstrated within the tumor cells.

A comprehensive review of the literature on so-called congenital rhabdomyoma of the heart revealed 62 authentic cases of tumors of the heart that presented a characteristic vacuolated appearance on microscopic examination.

The cardiac tumors were seen chiefly in infants and the newborn, with 52 per cent of the patients dying in the first year of life and 86 per cent before they reached puberty.

The clinical symptoms were nonspecific, and in no case was the diagnosis made before death. Frequently the symptoms were due to associated lesions. Tuberous sclerosis was found in 50 per cent of the cases.

We suggest that the term "congenital nodular glycogenic tumors of the heart" should replace "congenital rhabdomyoma" until more is known about the nature of the lesions.

40 Melnikov-Razvedenkov, N. F. *Mosk. M. J.* (10 10) **9** 64, 1929.

41 Sikl, H. *Časop. lek. česk.* **64** 757, 1925.

42 Baruchev, S. K. *J. Rann. Detsk. Vozr.* **13** 473, 1933.

43 Lymburner, R. M. *Canad. M. A. J.* **30** 368, 1934.

44 O'Flynn, E., and Mackay, H. *Proc. Roy. Soc. Med.* **30** 1063, 1937.

45 Eliakis, M. *Ann. de med. leg.* **17** 815, 1937.

46 Stewart, T. D. *New Zealand M. J.* **39** 63, 1938.

# PRIMARY TUMOR OF THE HEART

REUBEN STRAUS, M D, AND REUBEN MERLISS, M D

LOS ANGELES

Reports of primary tumor of the heart appear infrequently in the literature. Because of the reluctance of many students of the subject to accept the diagnosis given in some of these reports, there has been a considerable variation in the estimate of the number of cases that have occurred, the number varying with the bias of the author.

In 1938 Larson and Sheppard<sup>1</sup> accepted 155 cases as sufficiently authentic to be included in an introduction to the report of a case of their own. Since then only 7 more cases have been described.<sup>2</sup> We now add 3 additional ones that were recently encountered at autopsies, 2 on two successive days and the third nine months later.

## REPORT OF CASES

**CASE 1**—L. L., a 57 year old white man admitted to the Cedars of Lebanon Hospital on Dec 31, 1943, complained of having had difficulty in breathing, swelling of the ankles and the abdomen and considerable weakness for the preceding five to six months. He asserted that until the onset of his present symptoms he had been completely well and that his symptoms developed gradually and then became progressively worse. He further recalled that three months prior to admission he had become jaundiced for a week, but at no time were acholic stools noted. He complained of a lack of appetite and of a loss of 35 pounds (15.9 Kg) in weight. Concomitant with the onset of symptoms, he noted a pronounced reduction in urinary output, which persisted. The past history by systems contributed nothing of interest.

The patient appeared moderately cyanotic, tired and weak. The head and neck showed nothing remarkable. The chest revealed fremitus impaired, the percussion note dull and breath sounds suppressed posteriorly at the base of the right lung. A few crepitant rales were heard at the base of the left lung. The heart was normal in size by percussion, the pulse rate was 84 and the blood pressure 118 systolic and 110 diastolic. The cardiac tones were distant, but a rough systolic murmur

could be heard over the apex. The peripheral blood vessels were moderately sclerotic. There were moderate ascites and edema of the lower extremities. The venous pressure was 280 mm of water, and the circulation time with saccharine was nineteen seconds. The rest of the physical examination revealed nothing of importance.

The only significant laboratory findings were a low level of the blood cholesterol (109 mg per hundred cubic centimeters), slight polycythemia (erythrocytes 5,460,000 per cubic millimeter and hemoglobin of 15.7 Gm per hundred cubic centimeters), deficient plasma prothrombin time (40 per cent of normal), deficient removal of sulfobromophthalein from the plasma (90 per cent of the dye remaining in the blood one hour after injection), poor excretion of phenolsulfonphthalein (a trace excreted by the kidneys in two hours) and a urine volume of 170 cc for twelve hours, containing large numbers of leukocytes, erythrocytes, casts and a moderate amount of albumin.

The condition was diagnosed as a hepatorenal syndrome, but the picture was not clear. A thoracentesis of the right side of the chest yielded 1,000 cc of clear amber fluid.

During his stay in the hospital, the respiratory rate remained between 30 and 40. Cyanosis persisted, only temporarily relieved by oxygen. Terminally the pulse rate, which had been normal, rose to 120. The temperature stayed within normal limits except just before death, when it rose to 102 F. On the fourth hospital day the patient suddenly became markedly cyanotic and pulseless, and died.

**Autopsy** (about twenty hours after death).—The body was well nourished. There was pronounced cyanosis of the lips and the nail beds. No edema of the lower extremities was noted, but there was moderate edema of the subcutaneous tissue of the torso, as well as about 10 liters of clear serous fluid in the peritoneal sac, 2,000 cc in the left pleural sac, 500 cc in the right pleural sac and about 225 cc in the pericardial sac. The heart weighed 420 Gm. The external aspect presented nothing remarkable. When the heart was opened, the right atrium was considerably dilated. Attached to this aspect of the interatrial septum just posterior to and above the foramen ovale was an ovoid polypoid mass, 10.5 by 6 by 3.5 cm, about one third of which projected into the right ventricle. A smooth glistening membrane covered this mass. It was soft, semitranslucent and mottled yellow, reddish brown and grayish white. On section it presented an extremely fine glistening texture, due in part to edema. The pedicle was short, measuring 1.5 cm in length. At the point of attachment to the atrium the substance of the stalk blended with the subendocardial stroma. The foramen ovale was patent to a probe along its posterior margin. The endocardium, the valve leaflets and the myocardium presented nothing remarkable except for moderate hypertrophy of the muscularis of the right atrium and ventricle. No mural thrombi were seen.

Microscopic examination of the cardiac tumor revealed a relatively acellular eosinophilic matrix, in

From the Department of Pathology of the Cedars of Lebanon Hospital.

1 Larson, C. P., and Sheppard, J. A. *Arch Path* 26:717, 1938.

2 (a) Bennett, D. W., Konigsberg, J., and Dublin, W. *Am Heart J* 16:117, 1938. (b) Boman, P. G. *Ann. Int. Med.* 12:258, 1938. (c) Haythorn, S. R., Ray, W. B., and Wolff, R. A. *Am J Path* 17:261, 1941. (d) Jackson, M. N., and Jacobson, J. N. *Lancet* 2:740, 1939. (e) Martin, W. C., Tuohy, E. L., and Will, C. *Am Heart J* 17:728, 1939. (f) Reisinger, J. A., Pekin, T. J., and Blumenthal, B. *Ann Int Med* 17:995, 1942. (g) Strouse, S. *Arch Int Med* 62:401, 1938.



part granular and in part hyaline, in which were seen numerous well developed blood vessels and scattered round, spindle and stellate cells. In a few areas there was considerable extracellular and intracellular hemosiderin, the latter within endothelial phagocytes. Muci-

creased venous pressure noted clinically. The lungs presented moderate congestion, edema and focal atelectasis.

The spleen weighed 300 Gm and showed considerable chronic passive congestion. The liver weighed 1,940

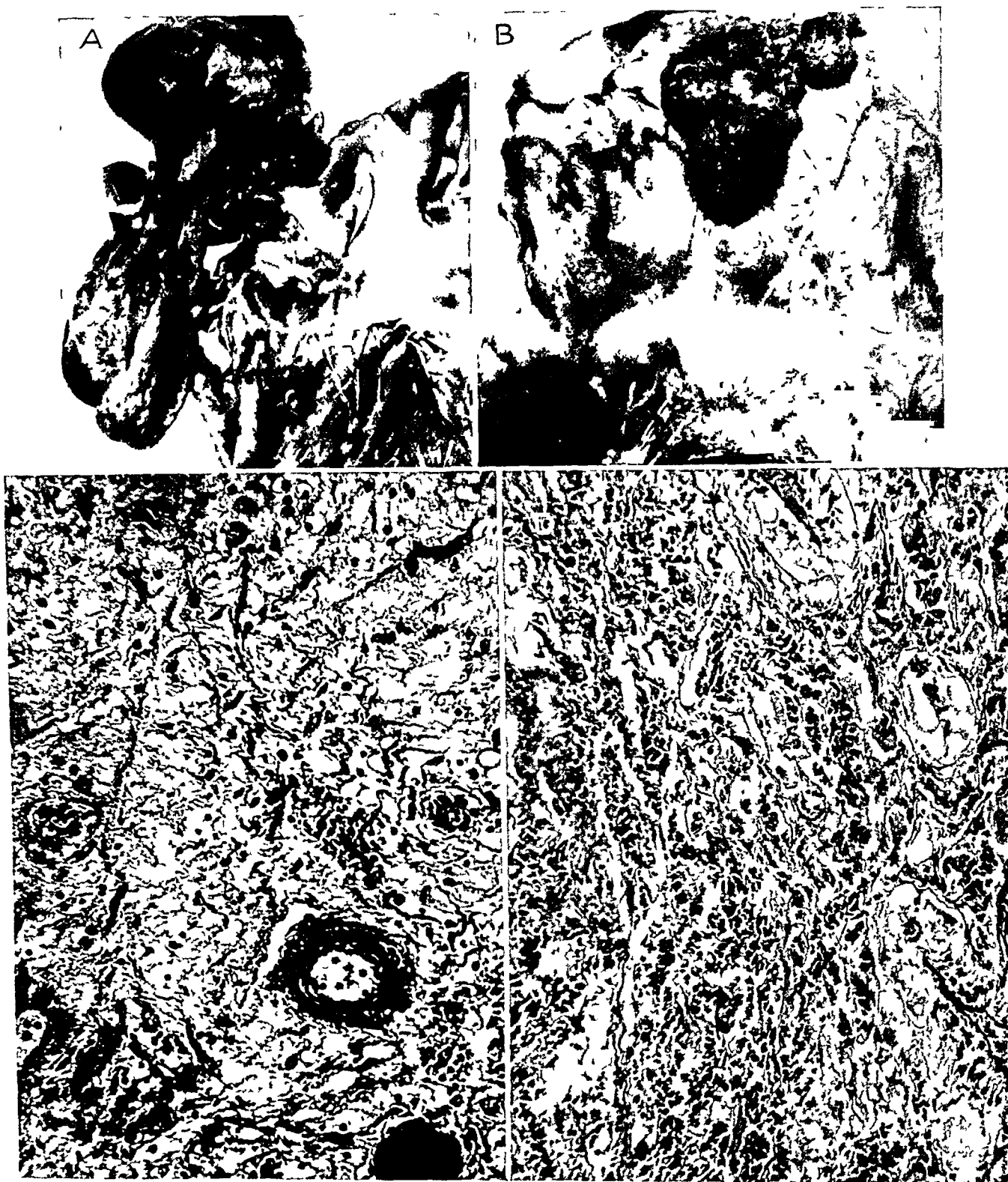


Fig 1—*A*, myxoma in case 1 elevated to reveal its attachment to the interatrial septum. *B*, primary myxosarcoma of the left atrium in case 2. *C*, section of the tumor in case 1 (hematoxylin and eosin). *D*, section of the tumor in case 2 (hematoxylin and eosin).

carmine stains showed essentially no mucin, only a faint red discoloration being noted in areas, which could have been artefact. With Wilder's reticulum stain sections revealed abundant reticulum.

The walls of the inferior vena cava and of the splenic vein were considerably thickened, reflecting the in-

creased venous pressure noted clinically. The lungs presented moderate congestion, edema and focal atelectasis. The spleen weighed 300 Gm and showed considerable chronic passive congestion of a severe degree. The other organs of the abdominal cavity presented no significant abnormality.

Death in case 1 was due to the cardiac tumor, myxoma, which by a "ball and valve" mechanism



produced marked stenosis of the tricuspid valve with severe cor pulmonale and effusions in the serous cavities of the body

**CASE 2**—E N, a 62 year old white woman was admitted to the Cedars of Lebanon Hospital, Dec 24, 1943, in a disoriented state with a diagnosis of diffuse cerebral arteriosclerosis and psychosis. She died on the thirteenth hospital day with signs of pneumonia. Since there was no clinical evidence of cardiac abnormality, details of the history and the examination are omitted.

**Autopsy** (about nine hours after death)—The body was well nourished. There was no edema and no effusion in any of the serous cavities. The heart weighed 320 Gm. No abnormality was seen on the external

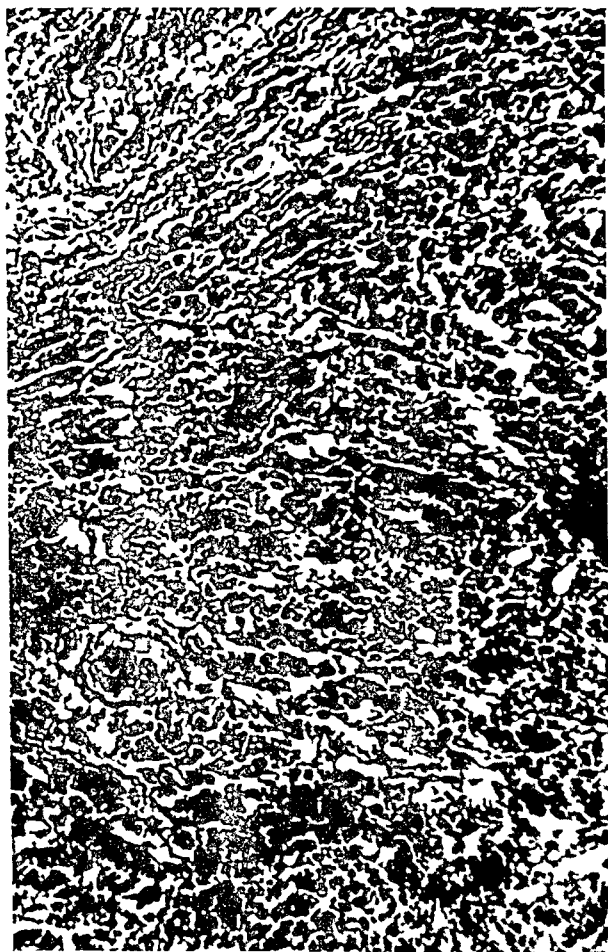


Fig 2—Section of the tumor in case 2 with reticulum stain

aspect. After the heart was opened, a fungating mass with a narrow pedicle was found attached to the left side of the interatrial septum just above the foramen ovale. It measured 5 by 4.5 by 3 cm. It was gelatinous, with hemorrhagic and friable distal portions. The cut surface revealed irregularly distributed white fibrous strands with some areas, chiefly at the base, which were yellow. The myocardium of the septum was not sharply demarcated from the stalk of the tumor. Embedded between the papillary muscle and the columna carnea of the left ventricle were two small gelatinous nodules, the larger 8 mm in diameter, which resembled the tumor of the atrium. On section superficial invasion of the myocardium could be seen. Except for patency of the foramen ovale along the posterior border, no other abnormality was found in the heart.

The lungs were heavy, considerably congested and edematous with patchy areas of bronchopneumonia in both lower lobes. The other thoracic and abdominal viscera revealed no significant abnormality. The brain weighed 1,220 Gm. There was severe arteriosclerosis of the arteries at the base. Repeated section revealed no evidence of encephalomalacia, old or recent.

Microscopic examination of the arterial tumor revealed homogeneous, translucent, in part hyaline and in part granular appearing matrix in which were embedded relatively large well formed vessels and scattered spindle, round and stellate cells. The tissue was suggestive of mucoid connective tissue. The tumor was considerably more cellular at the base than in the superficial portions but invaded the atrial wall only slightly. Large amounts of hemosiderin were visible in the hemorrhagic areas, some contained within endothelial phagocytes. Stains for mucin showed none. Abundant reticulum was demonstrated with Wilder's reticulum stain. Sections of one of the ventricular nodules presented a strikingly similar structure with slight invasion of one of the papillary muscles.

Sections from the lungs confirmed the gross findings of bronchopneumonia, congestion and edema. Sections of the brain, taken at random, failed to reveal striking variations from the normal. No metastatic tumor nodules could be found.

Death in case 2 was due to the cerebral arteriosclerosis with psychosis and bronchopneumonia. The cardiac tumor, classified as myxosarcoma because of the cellularity at its base, the infiltrative tendency and the implants in the left ventricle, was merely a coincidental finding.

**CASE 3**—F C, a 35 year old white man, was admitted to the Cedars of Lebanon Hospital Aug 17, 1944. About three weeks earlier, while at work he observed bullous lesions of his hands developing, followed by a generalized pruritic erythematous macular eruption. About two weeks later abdominal cramps and bloody diarrhea made their appearance, associated with nausea and vomiting.

Examination revealed very severe shock, rapid respirations, stupor, abdominal tenderness and distention, and hemorrhagic pleural effusions. Laboratory tests showed evidence of extensive damage of the liver and the kidneys. His course in the hospital was rapidly downhill, death occurring on the third hospital day.

**Autopsy** (about fourteen hours after death)—The hemorrhagic ulcerative enteritis, the severe cloudy swelling of the liver and the kidneys and the hemorrhage of the skin and the lungs were suggestive of poisoning, probably industrial in origin. Chemical examination revealed only slight amounts of cadmium in these organs. The paralytic ileus with peritonitis and bronchopneumonia were terminal complicating events.

The incidental observation of a tumor of the heart made this organ of special interest. It weighed 380 Gm. The external aspect was not remarkable. On the right side of the interatrial septum, situated 1 cm above the obliterated foramen ovale was a pedunculated papillary tumor nodule 1 cm in diameter. The tumor was tan, semitranslucent, with a fine fibrillar structure, and was attached to the endocardium by a thin delicate stalk. The valve leaflets, the endocardium and the myocardium presented no abnormality.

Microscopic examination of the tumor revealed a villus structure covered by a prominent layer of endothelium. The stroma was homogeneous, relatively acellular and hyaline, in places deeply acidophilic and in part lavender. A few scattered small mononuclear

cells could be seen. The lesion was classified as papillary myxoma.

## COMMENT

Historically,<sup>3</sup> Boneti in 1700 and Morgagni in 1762 have both been credited with having been the first authors to describe primary tumors of the heart. However, many pathologists believe that Albers in 1835 reported the first authentic case of primary tumor of the heart, fibroma, and that Bodenheimer<sup>4</sup> in 1865 described the first authentic primary sarcoma of the heart. Much of the early difficulty in assigning these credits was due to the inability to distinguish convincingly between a thrombus and a tumor.

Perlstein,<sup>3</sup> Goldstein,<sup>5</sup> Morris,<sup>6</sup> Yater<sup>7</sup> and more recently Strouse<sup>2g</sup> have presented excellent reviews of the entire subject of primary tumor of the heart. Briefly, it is stated that the site of tumor may be in practically any part of the heart but that it is less frequently on a valve and most commonly in an atrium. Apparently the tumor may be either single or multiple. It is agreed that in all cases of tumor of the heart the neoplasm is of mesoblastic origin and therefore it has been reported as myxoma, fibroma, lipoma, lymphangioendothelioma, hemangioendothelioma, leiomyoma, rhabdomyoma, rhabdomyosarcoma, leiomyosarcoma, fibrosarcoma and polymorphous cell sarcoma. According to Yater,<sup>7</sup> the primary cardiac tumor is cancerous in 17.8 per cent of the cases, but more recently Larson and Sheppard<sup>1</sup> calculated the incidence of cancer to be as high as 29 per cent. According to the literature, the disease occurs twice as frequently among males as among females. The younger age group is chiefly affected, but ages have ranged from 10 months<sup>8</sup> to 79 years.<sup>3</sup>

Most authors reporting cases of tumor of the heart fail to make note of the incidence of the primary tumor in their autopsy experience and those who do give widely divergent observations. For instance, Shelburne<sup>9</sup> reports 3 cases of primary cardiac tumor among 1,200 autopsies at Baylor University, an incidence of 0.25 per cent while Lymburner<sup>8</sup> found 4 cases among 8,550 autopsies at the Mayo Clinic, an incidence of 0.05 per cent. On the other hand, none was observed by Scott and Garvin<sup>10</sup> in 11,000 autop-

sies at the Cleveland City Hospital, and Polla and Gogol<sup>11</sup> encountered not a single instance of cardiac tumor in the 12,000 autopsies at the Los Angeles County Hospital up to 1936. To date, practically 30,000 autopsies have been made at the last-named institution with the same negative experience. At the Cedars of Lebanon Hospital, approximately 1,550 autopsies have been made to date, in 3 of which primary tumor of the heart was observed, giving an incidence of 0.19 per cent. This wide variation would suggest that perhaps estimates of the incidence of this disease are erroneous for lack of a sufficient number of cases. From 1938 to 1942, according to statistics of the American Medical Association,<sup>11</sup> there were 480,331 autopsies in the United States. During this period 8 cases of primary cardiac tumor were reported. If it could be assumed that all such cases noted at autopsy were reported, and from the unusual nature of the lesion it appears quite likely they would be, the incidence would be 0.0017 per cent, which seems more consistent with the rarity of the lesion. This makes the occurrence of 3 instances of this rare condition at one institution within a short time (2 on consecutive days and 1 nine months later) a striking coincidence.

As a rule, tumor of the heart is not diagnosed clinically. In patients, who have a primary cancer elsewhere and subsequently show signs of cardiac irregularity, metastatic tumor of the heart has been diagnosed. On the other hand, there are many cases of metastatic tumor of the heart that present no electrocardiographic or clinical signs.<sup>10</sup> How such experience may lead to inaccurate though logical diagnosis is exemplified in a case reported by Beck and Thatcher.<sup>12</sup> Biopsy of metastatic tumors of the skin revealed spindle cell sarcoma. Later, when abnormal cardiac manifestations were noted, a diagnosis of metastatic sarcoma of the heart was made. At autopsy this was found to be primary sarcoma of the heart with generalized metastases.

Only 2 cases are recorded in which primary tumor of the heart was diagnosed before death. Shelburne<sup>9a</sup> cited Goettel<sup>13</sup> as having credited Pavlowsky<sup>14</sup> with being the first to make such a clinical diagnosis. However, Strouse, on reviewing Pavlowsky's original article, reported the citation to be erroneous. In Pavlowsky's case a correlation between the clinical and the postmortem observations was made only after a peduncu-

3 Perlstein, L. *Am J M Sc* **156** 214, 1918

4 Bodenheimer, cited by Larson and Sheppard<sup>1</sup>

5 Goldstein, H. I. *New York M J* **115** 97 and 158, 1922

6 Morris, L. M. *Am Heart J* **3** 219, 1927

7 Yater, W. M. *Arch Int Med* **48** 627, 1931

8 Lymburner, R. M. *Canad M A J* **30** 368, 1934

9 Shelburne, S. A. (a) *Ann Int Med* **9** 340, 1935,

(b) *Texas State J Med* **31** 433, 1935

10 Scott, R. W., and Garvin, C. F. *Am Heart J* **17** 431, 1939

11 Polla, J. A., and Gogol, L. J. *Am J Cancer* **27** 329, 1936

12 Beck, C. S., and Thatcher, H. S. *Arch Int Med* **36** 830, 1925

13 Goettel, L. *Deutsche med Wchnschr* **45** 937, 1919

14 Pavlowsky, R. *Berl klin Wchnschr* **32** 393, 1895

lated benign tumor of the right atrium with stenosis of the tricuspid valve was demonstrated at autopsy

Barnes and co-workers<sup>15</sup> in 1934 reported the case of a 62 year old woman in which a diagnosis of primary sarcoma of the heart was made tentatively during life from the finding of a hemorrhagic pericardial effusion, an electrocardiogram showing interference with the conduction system of the heart and a biopsy of a nodule in the shoulder girdle, diagnosed as metastatic sarcoma. At autopsy their contention was verified by the observation of a tumor which involved the right ventricle and auricle, diagnosed as rhabdomyosarcoma

Shelburne<sup>9a</sup> deserves praise for the scholarly analysis that is contained in his case report. His patient, a 24 year old Negro man, complained of abdominal pain, distention, fainting spells and cough and presented signs of right-sided cardiac failure of sudden onset and pericardial effusion. The diagnosis of primary tumor of the heart was based on (1) signs of cardiac decompensation, (2) presence of a serosanguineous pericardial effusion as shown by pericardial tap, with rapid recurrence, (3) absence of signs or symptoms of tuberculosis or syphilis, (4) absence of signs of cancer elsewhere, (5) exclusion of rheumatic fever and other infections by the nature of the fluid and the absence of fever and (6) exclusion of rupture of a cardiac aneurysm or a coronary vessel by a comparison of the erythrocyte count of the pericardial fluid with that of the peripheral blood. After his diagnosis was recorded, an electrocardiogram revealed a bundle branch block. At autopsy a tumor was found in the left atrium, infiltrating the atrioventricular septum half way to the apex of the heart, diagnosed as spindle cell sarcoma.

Whether or not primary tumor of the heart can be diagnosed prior to death will depend certainly on its ability to produce symptoms by interfering with the cardiac mechanism. A small, slowly growing tumor of a chamber of the heart, and in some cases a larger one in an "out of the way" position, would not be physiologically apparent. A tumor on a valve, if sufficiently large, could be expected to produce murmurs but, unless thought of, would certainly be overlooked in the differential diagnosis. A large tumor of an atrium with a ball and valve action in either the mitral or the tricuspid ring and with murmurs affected by a shift in the position of the body should certainly be amenable to clinical diagnosis if kept in mind. In regard to cancer, the signs and symptoms most likely to be produced would be hemorrhagic pericardial effusions due to peri-

cardial involvement, interference with the conduction system from an infiltration of the myocardium murmurs when the valves are involved, and if the tumor is friable, distant metastases and possibly emboli. At times the roentgenogram may be of assistance, particularly when a large bulky tumor produces gross changes in the shape of the heart or an intravenously injected radio-opaque dye outlines the chambers of the heart. All cardiac tumors are usually characterized clinically by a sudden onset of severe cardiac failure without any apparent etiologic or anatomic basis, and a fairly rapid downhill course. Strouse's case<sup>25</sup> of forty-three years' duration is the longest on record.

#### SUMMARY

Primary tumor of the heart occurs infrequently. Only 163 cases have been reported to date. Individual experience varies considerably, since some whose experience included as many as 30,000 autopsies have seen none whereas others have reported 3 cases of primary cardiac tumor among 1,200 autopsies. When the 8 cases of cardiac tumor reported from 1938 to 1942 in this country are viewed against the background of the national autopsy experience of 480,331 cases reported by the American Medical Association,<sup>16</sup> the incidence of primary tumor of the heart is 0.0017 per cent, assuming all cases to have been reported.

Of the 3 additional cases of primary tumor of the heart herewith reported, 2 were instances of myxoma and 1 was a case of myxosarcoma. In 2 the tumor was silent, in the third it produced clinical signs and symptoms of tricuspid stenosis.

As a rule, primary tumor of the heart is clinically silent. Only 2 cases are on record which were diagnosed prior to death. In both the tumor was a cancer involving the conduction system and in both there was a hemorrhagic pericardial effusion. In no case of benign tumor has the diagnosis been made before death.

The sudden onset of signs of cardiac decompensation in a young person without a preexisting history of cardiac disease, with no discoverable anatomic basis for the cardiac disease, a hemorrhagic pericardial effusion, sometimes an involvement of valvular function or of the conduction system and signs of valvular stenosis which change on motion of the body, as well as signs of mediastinal tumor with alteration of the appearance of the shape of the heart in the roentgenogram, should cause one to consider primary tumor of the heart in the differential diagnosis of the condition under examination.

<sup>15</sup> Barnes, A. R., Beaver, D. C., and Snell, A. M. *Am Heart J* 9:480, 1934.

<sup>16</sup> Necropsy Performance in Internship Hospitals, *J A M A* 110:974, 1938, 112:924, 1939, 114:1171, 1940, 116:1068, 1940, 118:1065, 1942, 120:852, 1943.

# ESOPHAGEAL CARCINOMA IN BRITISH WEST INDIAN AND PANAMANIAN NEGROES

A STUDY OF THE INCIDENCE, ETIOLOGIC FACTORS AND PATHOLOGIC  
ANATOMY IN FIFTY CASES

MAJOR WRAY J TOMLINSON

AND

FIRST LIEUTENANT LESTER A WILSON JR

MEDICAL CORPS, ARMY OF THE UNITED STATES

In recent reports<sup>1</sup> of esophageal carcinoma it has been suggested that these tumors are far more frequent in white males, especially in those of Jewish extraction. It is the purpose of this paper to present the data from complete autopsies in 50 cases of esophageal carcinoma occurring in Negroes, Indians and mestizo persons of British West Indian and Panamanian races.

## MATERIAL

The material for this study was obtained from the autopsy records of the institution with which we are associated and includes 50 histologically proved cases of carcinoma of the esophagus in the races under study. The basic data in these 50 cases are presented in tables 1 and 2.

## RESULTS AND COMMENT

For statistical purposes the British West Indian and Panamanian autopsy populations can be considered here to be Negroes as shown by the finding of the sickling trait in 11 per cent of both races at autopsy.

*Race, Sex and Age*—The racial distribution of esophageal carcinoma is shown in table 1. The marked preponderance of British West Indians is partially occasioned by the age group distribution of the autopsy populations,<sup>2</sup> more than half of the Panamanian autopsy population falling into the group in which the ages are under 41 years, in contrast to the British West Indian group. The sex and the age incidence are consistent with other reports.<sup>1</sup>

*Incidence*—The percentage incidence for the entire 50 cases cannot be determined since some of these cases were obtained from the autopsy index system and no information is available covering the entire autopsy series in regard to

the distribution by race, sex and age. However, figures are available for a fifteen and a half year period. In those statistics the British

TABLE 1—*Data on the Distribution by Race, Sex, Age Group and Locale and the Gross Appearance of the Tumor in Fifty Cases of Esophageal Carcinoma*

	Cases
Race	
British West Indian	49
Panamanian	1
Sex	
Males	45
Females	5
Age group	
21 to 30	1
31 to 40	6
41 to 50	13
51 to 60	12
61 to 70	13
70 and over	5
Location of tumor	
Upper third	5
Middle third	25
Lower third	22
Gross appearance	
Flat	25
Polypoid	25
Ulcerated	46
Diverticular	2
Submucosal extension	14
Gross penetration	38

West Indian male autopsy population totaled 1,564, and of these 35, or 2.3 per cent, had esophageal carcinoma. In the female autopsy population of 591 cases there were 5 cases of esophageal carcinoma or less than 1 per cent. In the same statistics 197 British West Indian males had carcinoma, in 35, or 17.7 per cent, of whom it was in the esophagus. Similarly among the females there were 80 with carcinoma in 5, or 6.3 per cent, of whom it was in the esophagus. In the same statistics the cases of esophageal carcinoma in males were exceeded in number only by the cases of gastric carcinoma (20 per cent) and those of prostatic carcinoma (18.3 per cent).

*Etiologic Factors*—No conclusive evidence concerning the etiologic agent, or agents, pro-

1 Hollinger, P. H., and Hare, H. J. *Laryngoscope* 52:968, 1942. Vinson, P. P. *Northwest Med* 32:320, 1933. Mathews, R. W., and Schnabel, T. G. *J. A. M. A.* 105:1591, 1935.

2 Tomlinson, W. J., and Wilson, L. A., Jr. *Cancer Research*, to be published.

ducing esophageal carcinoma was found. There were no incidental ulcers of the esophagus in the entire series. Leukoplakia was present in 2 instances, with syphilis in 1 and with no demon-

TABLE 2—Autopsy Data in Fifty Cases of Esophageal Carcinoma

Cell type	
Squamous cell carcinoma	18
Adenocarcinoma	2
Grade of malignancy	
Grade 1	3
Grade 2	20
Grade 3	16
Grade 4	11
Direct gross extension to	
Trachea	33
Mediastinum	24
Right bronchus	17
Left bronchus	11
Aorta	10
Right pleura	10
Left pleura	8
Stomach	5
Diaphragm	4
Larynx	2
Pericardium	2
Hemorrhage from erosion of	
Esophageal veins	2
Common carotid artery	1
Metastases to	
Lungs	10
Liver	9
Ribs and vertebrae	7
Kidneys	2
Pancreas	1
Spleen	1
Peritoneum	1
Lymph nodes	
Esophageal	50
Bronchial	21
Tracheal	20
Preaortic abdominal	9
Cervical	4
Thoracic vertebral	4
Gastric	4
Thoracic aortic	2
Supraclavicular	2

strable evidence of syphilis in 1. Leukoplakia and syphilis have both been considered as possible causative factors of esophageal carcinoma. That syphilis may be a factor is strengthened by

the presence of definite clinical and autopsy evidence of syphilis in 26 instances, or 52 per cent, while in 22 cases, or 44 per cent, there was no evidence of this disease at the time of autopsy, and in 2 instances a decision could not be made. The average incidence of syphilis in the autopsy population under consideration for a ten year period as determined by clinical and autopsy data was 87 per cent.

In 2 cases the carcinoma was definitely recognized as having originated in a diverticulum, and it is possible that in cases of advanced or perforating carcinoma the neoplasm may have destroyed a preexisting diverticulum.

*Pathologic Anatomy*—The observations at autopsy in these cases are consistent with other reports dealing with large series of cases,<sup>1</sup> and no detailed analysis of the material presented in tables 1 and 2 will be made beyond calling attention to the constant involvement of the periesophageal lymph nodes by the carcinoma in all cases.

#### SUMMARY

In British West Indian Negroes, carcinoma of the esophagus ranks third as to number of cases among all the types of carcinoma observed at autopsy.

Of the 50 persons with esophageal carcinoma, 26 had clinical and autopsy evidence of syphilis and 22 had no recognizable syphilis. A decision could not be made in regard to the remaining 2. The average incidence of syphilis in the entire autopsy population for the past ten years is 87 per cent.

In the 50 cases of esophageal carcinoma studied, the periesophageal lymph nodes were grossly invaded in every instance.

# EXPERIMENTAL HYPERTENSION

## ITS PRODUCTION IN DOGS BY INTRAVENOUS INJECTION OF STREPTOCOCCI

GEORGE F DICK, M D

CHICAGO

Much has been added to present knowledge of the mechanism of hypertension by the work of Goldblatt and his associates on the renal ischemia produced by placing clamps on the renal arteries. As Goldblatt<sup>1</sup> put it, "Whatever may be the nature of the actual disturbance of hemodynamics which occurs in the human kidney, the seat of arterio and arteriolar sclerosis, a similar effect on hemorenal dynamics is probably produced in animals by persistent constriction of the renal artery."

With regard to the causes of such hemodynamic disturbances, Longcope and Winkenwerder<sup>2</sup> and Weiss and Parker<sup>3</sup> and others have pointed out the importance of pyelonephritis. However, there is no proof that pyelonephritis accounts for hypertension in more than a small fraction of the instances of this condition, and the actual cause of what has been called essential hypertension and of the renovascular changes associated with the late stages of the disease is unknown. Christian<sup>3</sup> mentioned heredity as a factor.

Some years ago G. R. Dick and I<sup>4</sup> called attention to hypertensive renovascular disease following scarlet fever. Earlier we<sup>5</sup> had observed that the urine of patients with nonsuppurative nephritis frequently contained streptococci. The urine of normal persons, on the other hand, was either sterile or contained a few organisms which are common inhabitants of the normal urethra, namely, staphylococci or diphtheroids. We also observed that after acute disturbances of focal infection, such as the extraction of a tooth, the

number of streptococci in the urine increased. We<sup>6</sup> showed that during the severe angina in scarlet fever green-forming streptococci frequently gained entrance to the blood stream. More recently, we<sup>7</sup> described arterial lesions following the intravenous injection of streptococci in rabbits. I have made cultures of the urine of patients with essential hypertension and of patients with acute and chronic glomerular nephritis with hypertension and have been impressed by the frequency with which streptococci, usually *Streptococcus viridans*, but sometimes hemolytic streptococci, were found in the urine even when urinalysis gave no indication of renal disease.

The reports of Richards,<sup>8</sup> O'Kell and Elliott,<sup>9</sup> Hopkins,<sup>10</sup> Deshon,<sup>11</sup> Murray and Moosnick,<sup>12a</sup> Faillo,<sup>13</sup> Gudesteu Medeiros,<sup>14</sup> Geiger<sup>15</sup> and Murray and Moosnick<sup>12b</sup> on arterial blood cultures all indicate that bacteria gain entrance to the blood stream far more often than has been commonly supposed.

What is the result of the subjection of the body to constantly repeated invasion by bacteria in amounts insufficient to produce clinical evidence of infection?

It was to answer this question that the following work was undertaken.

### EXPERIMENTAL PROCEDURE

Streptococci from various sources were injected intravenously into dogs over considerable periods. The dogs selected for these experiments were young animals which could be trained to lie quietly on a board for

6 Dick, G. F., and Henry, G. R. *J. Infect. Dis.* **15** 176, 1914.

7 Dick, G. F., and Leiter, L. *Tr. A. Am. Physicians* **54** 87, 1939.

8 Richards, J. H. *J. A. M. A.* **99** 196, 1932.

9 O'Kell, C. C., and Elliott, S. D. *Lancet* **2** 864, 1935.

10 Hopkins, J. A. *J. Am. Dent. A.* **26** 2002, 1939.

11 Deshon, R. *Bol. Asoc. med. de Puerto Rico* **32** 92, 1940.

12 Murray, M., and Moosnick, F. *J. Lab. & Clin. Med.* (a) **26** 382, 1940, (b) **26** 801, 1941.

13 Faillo, P. S. *J. Dent. Research* **21** 19, 1942.

14 Gudesteu Medeiros. *Brasil-med* **56** 33, 1942.

15 Geiger, A. J. *J. Am. Dent. A.* **29** 1023, 1942.

From the Frank Billings Medical Clinic of the University of Chicago.

1 Lewis, H. A., and Goldblatt, H. *Bull. New York Acad. Med.* **18** 459, 1942.

2 Longcope, W. T., and Winkenwerder, W. L. *Bull. Johns Hopkins Hosp.* **53** 255, 1933.

3 Weiss, S., and Parker, F., Jr. *Medicine* **18** 221, 1939, cited by Christian, H. A. *Osler's Principles and Practice of Medicine*, ed. 15, New York, D. Appleton-Century Company, Inc., 1943, p. 937.

4 Dick, G. F., and Dick, G. R. *Scarlet Fever*, Chicago, The Year Book Publishers, Inc., 1938, p. 46.

5 Dick, G. F., and Dick, G. R. *J. A. M. A.* **65** 6, 1915.

blood pressure tracings, thus making anesthesia unnecessary. The dogs were kept in cages and maintained on a diet of meat and dog chow for a time so that control mean blood pressures could be taken, urinalyses made and the blood urea and nonprotein nitrogen determined. The blood pressure was recorded by means of a kymograph and a mercury manometer connected through a large glass cannula with a gage 18 needle, which was introduced by direct puncture into the femoral artery.

A 2.5 per cent sodium citrate solution was used as the anticoagulant in the system. The blood pressure tracings were generally run ten minutes with each arterial puncture to allow the blood pressure to be stabilized. All pressures have been recorded as the means of the systolic and diastolic pressures.

After the normal blood pressure of each animal had been established, the intravenous injections of streptococci from various sources were started. These injections were given for the most part five days in the week. The amount injected depended on the condition of the animal. An attempt was always made to give doses as large as could be injected without producing obviously harmful immediate effects. Usually the injections were of twenty-four hour broth cultures of organisms, or if a good growth took longer to develop, older cultures were used.

Specimens of urine for cultures were obtained under aseptic precaution by catheterization, and, in addition to plating, cultures were made by adding 2 cc of urine to 12 to 15 cc of ordinary nutrient broth with a few drops of blood added.

#### EXPERIMENTS

**CASE 1**—A W F was admitted to the urologic clinic of Albert Merritt Billings Hospital, April 1, 1937 with the complaint of swelling of both feet. Examination showed puffiness of the eyelids and pitting edema of both ankles. The blood pressure was 172 systolic and 94 diastolic, the urine contained albumin (1 plus), occasional cellular casts, and 7 to 8 red blood cells and 2 to 3 white blood cells per high power field, the blood urea clearance was 45, the urea content of the blood was 131 mg per hundred cubic centimeters, the Wassermann test was negative.

The patient gave a history of a sinus infection, with drainage and removal of nasal polyps two weeks before entering the hospital.

Cultures of material from the nose showed many hemolytic streptococci.

Oct 21, 1943 the patient again was seen in the urologic clinic, and the diagnosis of arteriosclerotic heart disease with hypertension was made. His blood pressure was 175 systolic and 118 diastolic. The urine showed no abnormality other than a trace of albumin.

Dog 31 (blood pressure 120, total nitrogen 9.98 Gm per liter, plasma protein 6.06 Gm, urea 172 mg and nonprotein nitrogen 28 mg per hundred cubic centimeters)—This dog was given intravenous injections of broth cultures of hemolytic streptococci from case 1, starting April 14, 1937. The beginning dose was 50 cc. The doses were increased to 100 cc and were injected five days a week until Oct 4, 1941.

June 14, 1937 the dog's blood pressure had risen to 172, October 21 the pressure was 148, Feb 16, 1938 it was 161, March 14, 1939, it had risen to 176. At the next determination, Jan 8, 1941, the pressure was 170, March 17 it was 170, September 12, 170, October 4, 190. Jan 13, 1942 the blood pressure was 190, February 12, 196, March 6, 190, April 9, 170.

The monthly urinalyses gave negative results until Jan 19, 1938, when a benzidine test showed a trace of blood. January 27 the urine was normal, but February

3 it showed a trace of albumin. Thereafter it was normal at monthly examinations until Oct 9, 1939, when there was a trace of albumin. At the monthly examinations thereafter it was normal until Aug 27, 1940, when there was a trace of albumin, it was normal thereafter until Feb 14, 1941, when there was again a trace of albumin. It was normal after this until April 4, 1941, when there was a trace of albumin. Some albumin was observed on several examinations after this, and on Nov 6, 1941 the albumin was 2 plus.

Chemical examination of the blood June 10, 1937, Dec 6, 1937, April 28, 1938 and Feb 9, 1940 gave normal results, but May 5, 1942, seven months after the injections were discontinued, the dog became sick and chemical examination of the blood gave urea 314 mg, nonprotein nitrogen 400 mg, albumin 4.39 Gm and globulin 4.44 Gm per hundred cubic centimeters.

The dog died at this time with pronounced uremia. An autopsy was made four hours later.

The kidneys were contracted kidneys of the arteriosclerotic type and together weighed 31 Gm. The capsule stripped with difficulty, being adherent throughout. The cortex was narrow and light in color. The heart was large and weighed 87.9 Gm without the contained blood. The wall of the left ventricle measured 7 to 9 mm in thickness, that of the right ventricle, 2 to 4 mm. The aorta and the coronary arteries showed no gross change. The lungs, the liver and the spleen showed no gross change. There was some light hemorrhage throughout the gastrointestinal mucosa.

Microscopically, the alterations were most pronounced in the renal cortex. There was an increase of fibrous tissue throughout the cortex, especially surrounding the glomeruli, the tufts of which were largely replaced by hyaline material and connective tissue. Blood vessels were thickened, and there were large areas showing lymphocytic infiltration.

**Summary**—Dog 31, with normal blood pressure and normally constituted blood, received intravenous injections of broth cultures of streptococci, a total of 32 liters, from April 14, 1937 to Oct 4, 1941. Hypertension developed in three months after the first injection. Five and one-half years after the first injection and seven months after the last injection the dog died of uremia with the arteriosclerotic type of contracted kidneys.

**CASE 2**—F H S, aged 42, entered the hospital Sept 15, 1941, complaining of disorientation, inability to talk, dyspnea on exertion and subcutaneous hemorrhages. The blood pressure was 210 systolic and 140 diastolic. The heart was 45 per cent oversize. The urine contained albumin (2 plus) and showed a hyaline cast, an occasional red blood cell, and 5 to 6 white blood cells per high power field. *Str. viridans* was demonstrated in the catheterized urine on culture. The Wassermann reaction was negative. The blood urea was 14.5 mg per hundred cubic centimeters. The blood urea clearance was 41. The diagnosis was malignant hypertension with some degree of cardiac failure.

Dog 92 (blood pressure 105, weight 10.2 Kg, urine normal)—This dog was given intravenously five times a week broth cultures of *Str. viridans* from the urine of case 2 from Nov 11, 1941 to Jan 2, 1942. This dog received a total of 2.56 liters. The urine on monthly examination remained normal. The course of the blood pressure is shown in figure 1. Fifteen months after the first injection the left kidney was removed for examination. It weighed 28 Gm and was a trifle lighter in color than normal. The capsule stripped readily except in a few places. The surface of the kidney showed a few arteriosclerotic depressions. Micro-



scopically, there was a slight but distinct increase in connective tissue in the blood vessels and especially in the glomerular capsules

CASE 3—D B, aged 51, entered the hospital Sept 3, 1942, complaining of driving pain in the back of the neck and head of three weeks' duration This came on

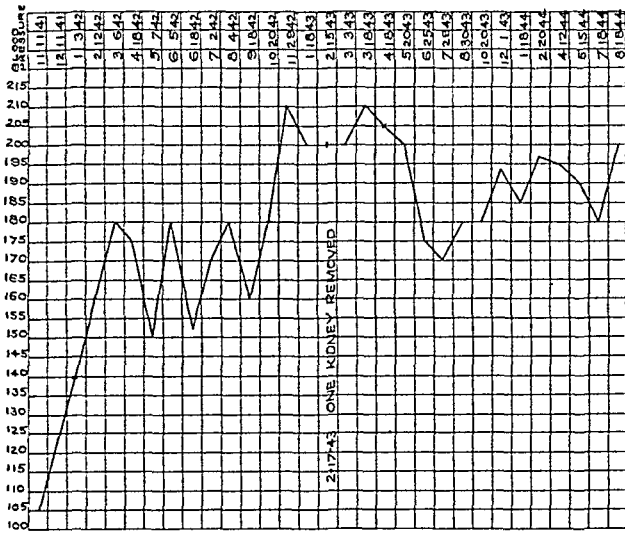


Fig 1—Blood pressure of dog 92 determined monthly

from 50 cc to 100 cc From Sept 25 to Dec 10, 1942, the dog received a total of 293 liters of the broth cultures intravenously October 20 the blood pressure had risen to 146, November 29, to 166 Feb 18, 1943 the blood pressure was 140, March 18, 174, April 9, 180 The blood urea Nov 18, 1943, seven months after the kidney was removed, was 99 mg per hundred cubic centimeters The urine showed no abnormality on monthly examination

April 12, 1943 the left kidney was taken out for examination It weighed 27 Gm and was normal except for two small areas that looked like retention cysts, about 1 mm in diameter Microscopically, it showed no abnormalities which could be made out on staining with hematoxylin and eosin, van Gieson or azan stains

Dog 147 represents what might be called essential hypertension, dog 92, hypertensive vascular disease with relatively slight arteriosclerotic changes in the kidney, dog 31, death from uremia with the arteriosclerotic type of contracted kidneys Photomicrographs of these kidneys (figs 2, 3 and 4) show the progressing changes in the microscopic appearance of the kidneys

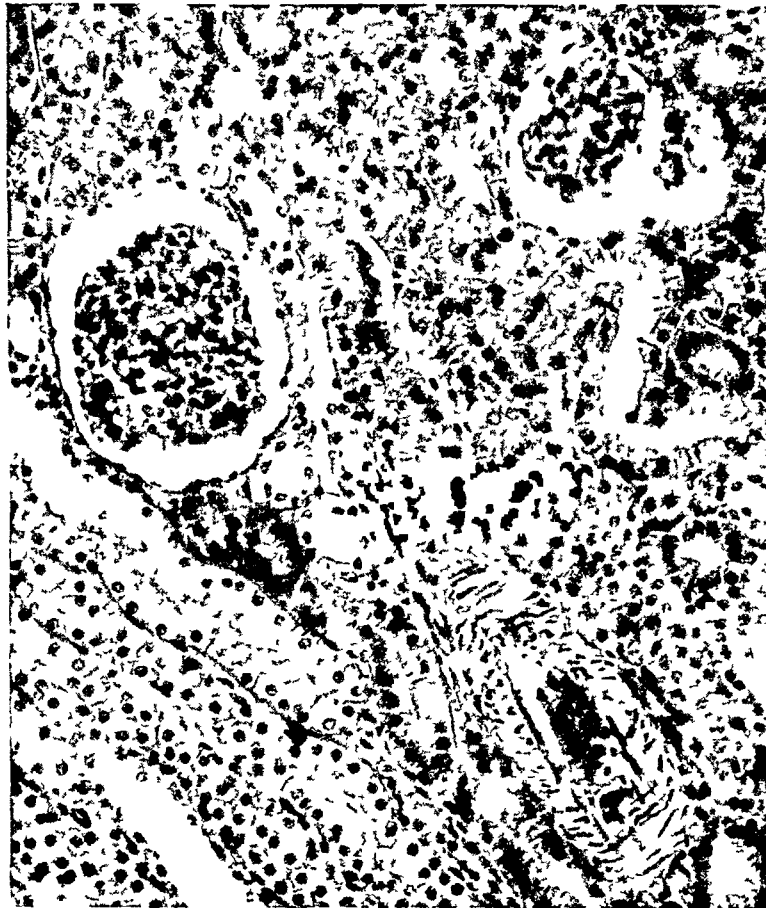


Fig 2—Photomicrograph of kidney of dog 147 showing no abnormality,  $\times 250$

suddenly after he had bowled four games The blood pressure was 230 systolic and 120 diastolic The urine showed no abnormalities *Str viridans* was isolated from the urine on culture The diagnosis was hypertensive vascular disease

Dog 147 (blood pressure 136, urine normal)—Broth cultures of *Str viridans* from case 3 were injected intravenously five days a week in amounts increasing

CASE 4—J B, aged 38, had hypertensive cardiovascular disease The blood pressure was 157 systolic and 118 diastolic Urinalysis gave normal results The blood urea was 21.2 mg per hundred cubic centimeters The blood urea clearance was 53

Dog 93—*Str viridans* was isolated from the catheterized urine in case 4, and broth cultures were injected into dog 93 in amounts gradually increased to 100 cc



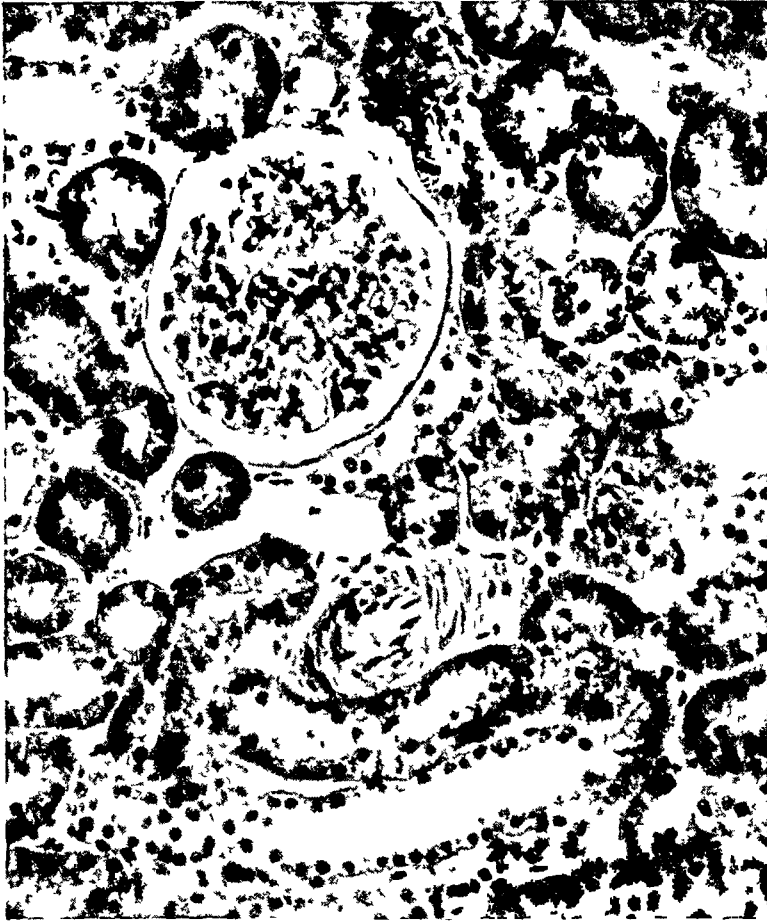


Fig 3—Photomicrograph of kidney of dog 92 showing slight increase in fibrous tissue,  $\times 250$

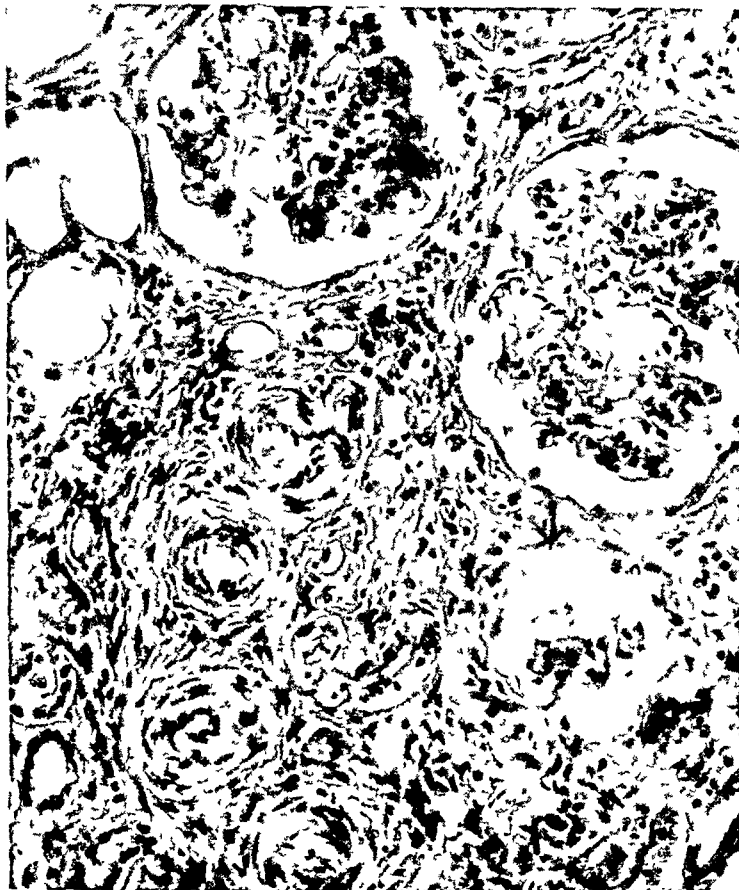


Fig 4—Photomicrograph of kidney of dog 31 showing the histologic picture of contracted kidney,  $\times 250$

a day The injections were continued as shown in figure 5 In three months the blood pressure had risen from 125 to 155 and in five months it had risen to 190

Dog 160—This animal received intravenous injections of cultures of the same organism, as dog 93, in approximately the same dosage Its blood pressure remained normal for six and one-half months, when the blood pressure rose to 170

CASE 5—E W M, aged 63, had hypertensive cardiovascular disease and arteriosclerotic heart disease The

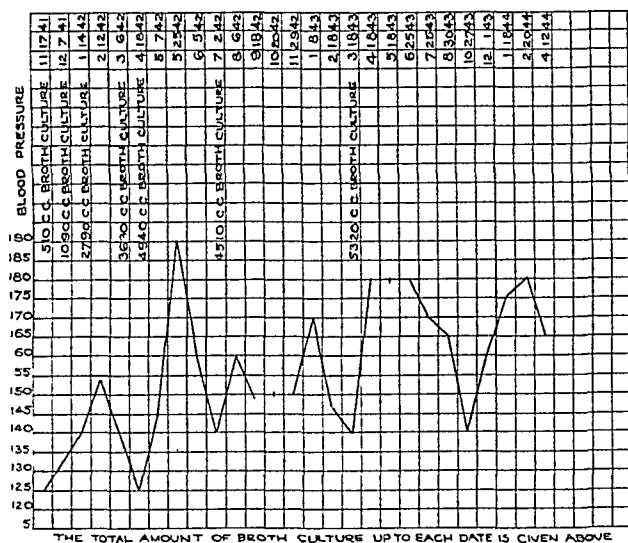


Fig 5—Blood pressure of dog 93, determined monthly The total amount of broth culture injected up to each date is given above

blood pressure was 236 systolic and 148 diastolic The urine contained albumin (3 plus), hyaline and granular casts, an occasional red cell and 4 to 5 white blood cells per high power field The blood urea was 11 mg per hundred cubic centimeters The blood urea clearance was 28 Str viridans was isolated from the catheterized urine

Dog 125—Broth cultures of the organism isolated in case 5 were injected intravenously into this animal, and the blood pressure rose from 120 to 155 in one and one-half months and to 180 in five months

CASE 6—P R, aged 16, had acute glomerular nephritis The blood pressure was 120 systolic and 70 diastolic The urine contained a trace of albumin and hyaline and granular casts Str viridans was isolated from the catheterized urine

Dog 36—Broth cultures of the streptococcus from the urine in case 6 were injected in increasing amounts up to 100 cc a day The blood pressure of the dog rose from 105 to 154 in three and one-half months It rose to 160 in six months, and in the seventh month the dog died of pneumonia The kidney showed slight arteriosclerotic scarring of the surface

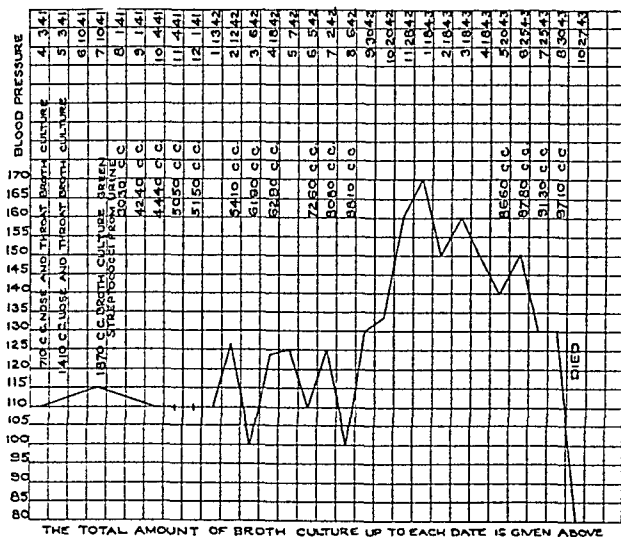
CASE 7—D H T, aged 25, was given a diagnosis of acute nephritis and acute pansinusitis The blood pressure was 116 systolic and 50 diastolic The urine contained albumin (2 plus), hyaline and granular casts, many red cells, and 10 to 12 white cells per high power field The patient had some fever, the temperature rising to 100 F Hemolytic streptococci were present in the discharge from the nose The blood urea was 23 mg per hundred cubic centimeters The urea clearance was 18 Str viridans was isolated from the catheterized urine

Dog 66—This animal was started with a whole broth culture of the hemolytic streptococci from the nose and throat in case 7, given in amounts increased to 100 cc, and later this culture was lost The injections were continued with cultures of the greenish streptococci from the urine, as shown in figure 6 The blood pressure rose from 110 to 160 in twenty months The dog died at the end of twenty-eight months On post-mortem examination there were some infarcts in both kidneys, and the cortex was thin and in part swollen with petechial hemorrhages The capsule stripped with some difficulty, leaving an irregular surface, mottled brownish gray and yellow The kidneys weighed together 55.5 Gm There were some areas resembling plaques of fibrous pericarditis (soldier spots) in the pericardium, and there was some hemorrhage in the heart muscle of the left ventricle The heart weighed 94 Gm The liver was very light in color

CASE 8—J R G, aged 2, suffered from a condition diagnosed as acute nephritis following tonsillitis The blood pressure was 130 systolic and 77 diastolic Str viridans was isolated from the catheterized urine The organism produced considerable hemolysis in contrast to other organisms described

Dog 158—The dog was given intravenous injections of the streptococci isolated in case 8 After receiving only 60 cc in three injections the blood pressure rose from 130 to 170 in nine days

CASE 9—B B, aged 63, was given a diagnosis of hypertensive cardiovascular disease with aortic stenosis The blood pressure was 220 systolic and 130 diastolic The blood urea was 94 mg per hundred cubic centimeters The urea clearance was 62 The urinalysis gave negative results Str viridans was isolated from catheterized urine and, like the streptococcus isolated in case 8, produced a wide zone of hemolysis as well as greenish



stripped readily. The cortex was not perceptibly narrowed. Hemolyzing, green-forming streptococci were grown from the kidney in pure culture.

Microscopically, there was not much change but there were areas of extensive plasma cell infiltration, some thickening of the arterioles and congestion of the glomeruli, with some red cells in Bowman's capsule. The cause of death in the animal was not clear. It was a pup about 4 months old when the injections were started.

CASE 10—R S, aged 57, had a diagnosis of coronary arteriosclerosis. The blood pressure was 148 systolic and 94 diastolic. The urinalysis gave negative results. A hemolytic streptococcus was isolated from the throat.

Dog 101—A broth culture of the hemolytic streptococcus isolated from the throat in case 10 was injected into this dog. In two months the blood pressure rose from 120 to 150, and two years later it was 160, three years later it was 185.

SUMMARY OF FURTHER EXPERIMENTS—Dogs 109, 1, 174 and 168 were all given injections of cultures of hemolytic streptococci from the throats of patients with glomerular nephritis. Further details will not be given in order to save space. The blood pressure of dog 109 rose from 120 to 165 in one month and to 185 in one year. Injections were given at intervals from Jan 5, 1942 to July 20, 1944. Dog 1 received injections from Feb 11, 1935 to Feb 1, 1939 and again from Aug 26, 1940 to Feb 8, 1941, when he died. The blood pressure remained normal for four years and then rose to 150, eight months later it was 170. At postmortem examination the dog was found to have endocarditis with vegetation along the entire mitral valve and hemorrhages in the myocardium. The kidneys showed embolic nephritis superimposed on an arteriosclerotic condition. There were hemorrhages in the mucosa of the gastrointestinal tract. The thoracic and abdominal portions of the aorta were normal. Hemolytic streptococci were isolated from the cardiac valve.

The blood pressure of dog 174 rose from 120 to 165 in two and one-half months. The blood pressure of dog 168 rose from 130 to 150 in six months.

In the experiments described all cultures were obtained from patients with renal-vascular disease. That streptococci from other sources act in a similar manner is shown by the following experiments.

Dog 104 was given injections of hemolytic streptococci from a patient with uncomplicated scarlet fever. After two months the blood pressure rose from 110 to 142 and in a year to 176. The dog died twenty-two months after the first injection and on autopsy showed bacterial endocarditis with embolic nephritis.

Dogs 135 and 105 were given injections of streptococci from 2 patients with erysipelas. The blood pressure of dog 105 rose from 130 to 150 in two months and to 160 in three months. The dog died in ten months, and at autopsy bacterial endocarditis was observed along with hemorrhagic pericarditis. The blood pressure of dog 135 rose from 120 to 140 in two months and to 155 in six months. The animal died eleven months after the first injection. The autopsy showed early bacterial endocarditis with embolic nephritis.

On account of the frequency with which hypertension follows chronic pyelonephritis, dogs 111 and 120 were given injections of colon bacilli. The blood pressure of dog 111 rose from 120 to 160 in one month and reached 184 in eleven months. The dog died seventeen months after the first injection. The autopsy showed hemorrhages in the peritoneal coat of the colon. The

kidneys weighed 50 Gm together. The capsule could not be stripped without pulling with it part of the cortex and leaving an irregular surface. The heart weighed 70 Gm and except for a pale myocardium showed no other change. The aorta was normal. Microscopically, the kidneys showed an increase in the fibrous tissue with some hyaline change. Many of the glomeruli were shrunken and contained what was probably albuminous material within Bowman's capsule. Pyelitis was present, and a large amount of infiltration with round cells. Many of the tubules were dilated and contained either hyaline or granular casts. The increase in fibrous tissue, however, was more apparent between the tubules and surrounding the glomeruli than within the walls of the arteries, which appeared to be normal.

Dog 120 was given injections of the strain of colon bacilli used in dog 111. In one month the blood pressure rose from 120 to 140, in twelve months, to 150. The dog died in twenty months after the first injection. No one was available to make the postmortem examination, but the kidneys were well preserved in Zenker's solution. Microscopically, there was considerable round cell infiltration surrounding the smaller blood vessels, and in some areas there was considerable increase in fibrous tissue. Pyelonephritis was present, similar to that found in dog 111. There was, however, some thickening of the walls of the arterioles in this kidney.

Dog 113 was given intravenous injections of a strain of *Str. viridans* isolated from the blood of a patient with subacute bacterial endocarditis. In one month the blood pressure had risen from 135 to 160, in ten months to 190, in two years to 196. Although injections were stopped after six months, the lowest monthly blood pressure obtained was 170.

Dogs 64 and 96 were both given intravenously a culture of staphylococci from a postoperative suppuration. The blood pressure of dog 64 rose from 100 to 145 in eight months and in nine months to 155. The dog died in fifteen months, one day after the last injection, after receiving a total of 8,340 cc of broth cultures of staphylococci. The autopsy showed petechial hemorrhages throughout the bowel and in the endocardium. Duodenal ulcers were present. The kidneys were somewhat light in color, the capsule stripped with some difficulty, and some of the cortical tissue was removed with it.

The blood pressure of dog 96 rose in one month from 130 to 160, in ten months to 165, in thirteen months to 180. From that time the monthly variations were large, with the pressure as low as 130 in one instance and 150 in several instances, in three years it was 180. This dog is still alive. The total amount injected was 5,430 cc.

It is apparent, then, that the intravenous injections of streptococci in dogs is followed by a rise in their blood pressure and that this happens whether the streptococci are isolated from patients with hypertension or from patients without hypertension or are from stock cultures. It happens with green-forming streptococci, with hemolytic streptococci, and with streptococci producing both greening and hemolysis on blood agar plates. How many other varieties of bacteria may produce hypertension in dogs we do not know. Forsbeck<sup>16</sup> stated that hypertension

is more likely to occur in typhoid carriers than others of the same age. Injections of colon bacilli were followed by hypertension as described. However, the lesions in the kidney were somewhat different from those produced by streptococci, especially in that pyelonephritis was present. With injections of staphylococci, also, hypertension was produced. With organisms other than *Str. viridans*, the pathologic picture produced is likely to be more complicated, and failure to produce hypertension is more common, owing to the fact that the animal dies shortly after the beginning of injections either from toxic effects or septicemia and pyemia with or without endocarditis. In general it seems easier to produce uncomplicated hypertension with less virulent organisms, since it is difficult to regulate the dose of the more virulent organisms so as to avoid fatal disease before or after hypertension has time to develop. Most commonly these fatalities were observed with organisms from subacute bacterial endocarditis.

It is of interest to note that in 9 dogs with hypertension the average weight of the heart was 8.51 Gm per kilogram of body weight, whereas the average weight of the heart in normal dogs was 7.25 Gm per kilogram.

That hypertension does not invariably follow intravenous injections of streptococci, even when the organisms are given in doses which do not cause these complications, is shown in dog 91. This dog was given injections of the same culture and at the same time as dog 92. While the blood pressure rose in dog 91 to 140, it never went higher, and at the end of three years and four months the dog died. At autopsy the kidneys weighed 67 Gm. They showed increased consistency and were lighter in color than normal. The capsule when stripped left a distinctly granular surface, with the nodules making up the surface varying in size to that of a rice grain. The heart weighed 81 Gm. Microscopically, the changes in the kidneys were similar to those described in the biopsy specimen of the kidney of dog 92. Figures 2 and 7 show the difference of the variations in blood pressure of these two animals.

In 8 other instances dogs received the same injections of streptococci as did others in which hypertension developed, and in the case of both green-forming and hemolytic streptococci no hypertension followed, although slight arteriosclerotic changes in the kidney were observed in 4. It is possible that in some of these dogs hypertension would have developed later.

The failure to produce hypertension in such instances gives rise to the question whether the increases in the instances in which the blood pres-

sure did rise after injections of streptococci were coincidences and represented spontaneous increases which would have occurred in the dogs whether bacterial injections were made or not. The question is the more pertinent because the percentage of dogs found with spontaneous hypertension is considerable. However, of the total number of dogs given injections in which immediate death, endocarditis, septicemia or other reasons prevented any chance of hypertension following, 31, or 73.4 per cent, presented hypertension, and 11, or 26.2 per cent, did not. Moreover, in selecting dogs for this work, of 99 dogs examined, only 20, or 20.2 per cent, had to be discarded on account of hypertension.

On account of this fact, it seems reasonably certain that the injections caused the following hypertension. It is not unlikely that the hyper-

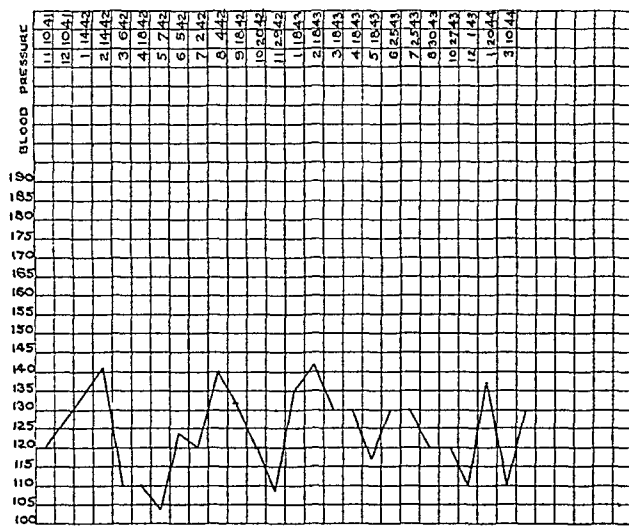


Fig 7—Blood pressure of dog 91, determined monthly

tension that occurred spontaneously in our dogs may have been due to infections. Many of the animals were found to have hemolytic streptococci in the throat, and it is well known that dogs frequently suffer from hemolytic streptococcal tonsillitis.

The next question arising is: Would the hypertension following injections of broth cultures have occurred had broth alone been used?

As long ago as 1913 Longcope<sup>17</sup> described renal lesions following injections of horse serum and egg albumin in dogs and rabbits. These lesions showed round cell infiltration, degeneration and connective tissue proliferation.

Dogs 161, 167 and 173 were given injections of sterile broth identical with that used in making the broth cultures employed in the preceding experiments, the same technic was used, but the broth was injected in larger quantities. Dog 161

<sup>17</sup> Longcope, W. T. *J. Exper. Med.* 18: 678, 1913.

received 7,000 cc of broth intravenously over a period of thirteen months. The initial blood pressure was 130. The blood pressure rose in five months to 160 but in eight months more was only 145.

Dog 167 received 6,190 cc of broth intravenously in ten months. At the time the injections were started the blood pressure was 140. It did not rise above this point, and ten months later was 135.

Dog 173 received 7,380 cc of broth over a period of ten months. The blood pressure rose from 125 to 155 in this time.

It will be seen that while some rise in blood pressure occurred following intravenous injections of broth, it was not great in degree, although large quantities of broth were used over a period in which much higher pressures were produced with smaller amounts of bacterial cultures.

#### CONCLUSION

Hypertensive states comparable to essential hypertension and hypertensive renovascular disease in man have been produced in dogs by intravenous injections of streptococci and other bacteria.

# OSSIFIED CARTILAGE WITH MYELOID FAT MARROW IN THE AORTIC RING OF A RABBIT

W C HUEPER, M D

NEW YORK

Vanzetti<sup>1</sup> and Hueper<sup>2</sup> have recorded the relatively frequent occurrence of hyaline cartilaginous plates of the aortic ring in rabbits. They considered these formations as the result of nonmoplastic metaplasia of fibrous tissue. Dratschinski<sup>3</sup> made similar observations but regarded the formations as pathologic reactions

advanced age. Hueper<sup>4</sup> recently recorded the occurrence of bone with myeloid marrow in a cartilaginous focus at the base of the aorta of a dog.

The present report deals with a similar lesion in the aortic ring of a rabbit which had received in its daily diet 0.25 Gm of cholesterol in oil and



A focus of cancellous bone surrounded by traces of cartilaginous tissue in the aortic ring of a rabbit. In the bone note marrow cavities filled with fat tissue and a scattering of immature myeloid cells.

The presence of cartilaginous plates in the aortic ring has been reported in other species, such as mice, rats, sheep, buffaloes, horses, turtles, alligators and chickens (Hueper<sup>2</sup>). Enchondral ossification of these foci was seen in sheep of

10 cc of a 1 per cent aqueous solution of the detergent Triton K-60<sup>4a</sup>. The aorta of this animal showed in the intima numerous cushions of foam cells and in the aortic ring a large focus of cancellous bone surrounded by traces of cartilaginous tissue. The marrow cavities of the bone were filled with fat tissue containing a

From the Warner Institute for Therapeutic Research

1 Vanzetti, F. Arch. ital. de biol. **56** 265, 1911

2 Hueper, W. C. Arch. Path. **27** 466, 1939

3 Dratschinski, cited by Gruber<sup>7</sup>

4 Hueper, W. C. J. Am. Vet. M. A. **101** 493, 1942

4a Triton K-60 is cetyledimethyl benzylammonium chloride (Rohm & Haas Co., Inc.)

moderate scattering of immature myeloid cells (figure)

This observation is of interest not only because of its rarity but also because cartilaginous and osseous tissue with myeloid marrow occurs in the walls of sclerotic aortas and arteries of rabbits and man. Harvey<sup>5</sup> recorded the presence of osseous tissue with marrow in the abdominal aorta of the rabbit following the painting of the outside of the vessel with a 3 per cent solution of silver nitrate or a 2 per cent solution of copper sulfate. Seegal and Seegal<sup>6</sup> noted the spontaneous formation of bone and marrow in the aorta of a rabbit. The incidence of myeloid tissue in aortic lesions of this type seems to be low, as cartilaginous foci in the aortic media are not infrequent in spontaneous arteriosclerosis in rabbits<sup>7</sup> and have been observed in the medial aortic lesions of rabbits induced by the injection of epinephrine hydrochloride.<sup>8</sup>

While ossification of calcified foci in the intima and the media of the sclerotic aorta and of the femoral, carotid and uterine arteries in man is not unusual,<sup>9</sup> the presence of myeloid tissue in

such lesions is apparently infrequent.<sup>10</sup> Freudenstein<sup>10d</sup> asserted that the appearance of hematopoietic tissue in the calcified vascular wall depends on the development of vascular structures invading the calcified matter. The myeloid tissue derived from these vessels precedes the ossification. Cartilaginous tissue either alone or in connection with osseous tissue is rarely encountered in the walls of human arteries and in those animals other than rabbits.<sup>11</sup>

#### SUMMARY

Metaplastic transformation of a cartilaginous plate in the aortic ring of a rabbit into cancellous bone with myeloid tissue was observed.

5 Harvey, W. H. *J. M. Research* **17** 1, 1907.  
6 Seegal, B. C., and Seegal, D. *Arch. Path.* **3** 73, 1927.

7 Gruber, G. B. *Virchows Arch. f. path. Anat.* **275** 541, 1930.

8 Miesowicz, E. *Centralbl. f. allg. Path. u. path. Anat.* **18** 8, 1907. Erb, W. *Arch. f. exper. Path. u. Pharmakol.* **53** 173, 1905. Trachtenberg, M. A. *Centralbl. f. allg. Path. u. path. Anat.* **17** 611, 1906. Otto, C. *Virchows Arch. f. path. Anat.* **203** 352, 1911. Studzinski, cited by Gruber.<sup>7</sup>

9 Monckeberg, I. G. *Virchows Arch. f. path. Anat.* **167** 191, 1902. Sappington, S. W., and Fisher, H. R. *Arch. Path.* **34** 989, 1942. Cohn, cited by Gruber.<sup>7</sup> Huebschmann, P. *Beitr. z. path. Anat. u. z. allg. Path.* **39** 119, 1906. Weizmann, M. *Systematische histologische Untersuchungen über den Ductus resp. das Ligamentum Botalli im Anschluss an einen Fall von Verknorpelung des letzteren*, Inaug. Dissert., Zurich, A. Schereschewsky, 1911. Jores, L. *Arterien*, in Henke, F., and Lubarsch, O. *Handbuch der speziellen pathologischen Anatomie und Histologie*,

Berlin, Julius Springer, 1924, vol. 2, p. 639. Kaufmann, E. *Lehrbuch der speziellen pathologischen Anatomie*, ed. 7 and 8, Berlin, Walter de Gruyter & Co., 1922, vol. 1, p. 78. Wolkoff, cited by Nieberle, K. *Verhandl. d. deutsch. path. Gesellsch.* **25** 291, 1930. Rohmer, P. *Virchows Arch. f. path. Anat.* **166** 13, 1901. Ceelen, cited by Ropke. *Klin. Wchnschr.* **11** 921, 1932. Bunting, C. H. *Folia haemat.* **3** 244, 1906. J. *Exper. Med.* **8** 365, 1906. Burger, L., and Oppenheimer, A. *J. Exper. Med.* **10** 354, 1908. Howse, H. G. *Tr. Path. Soc. London* **28** 90, 1877. Marchand, M., in Eulenburg, A. *Real-Encyclopedie der gesamten Heilkunde*, ed. 3, Leipzig, Urban & Schwarzenberg, 1894, vol. 2, p. 203. Orth, J. *Lehrbuch der speziellen pathologischen Anatomie*, Berlin, A. Hirschwald, 1883, vol. 4, p. 225. Bensen, G. *Beiträge zur Kenntnis von der heteroplastischen Knochenbildung*, Inaug. Dissert., Göttingen, 1898. O'Brien, L. J. *Ueber Verknöcherungsvorgänge in den Arterien*, Inaug. Dissert., Wurzburg, C. J. Becker, 1902. Poscharissky, cited by Gruber.<sup>7</sup>

10 (a) Borchardt, H. *Virchows Arch. f. path. Anat.* **259** 373, 1926. (b) Edelmann, A. *ibid.* **266** 51, 1927. (c) Thiersch, H. *Beitr. z. path. Anat. u. z. allg. Path.* **96** 147, 1935-1936. (d) Freudenstein, M. *Frankfurt Ztschr. f. Path.* **2** 591, 1909. (e) Rosenstein, P. *Virchows Arch. f. path. Anat.* **162** 100, 1900.

11 Marburg, O. *Centralbl. f. allg. Path. u. path. Anat.* **13** 300, 1902. Lillie, R. D. *Arch. Path.* **18** 710, 1934. Spiegl, A. *Virchows Arch. f. path. Anat.* **231** 224, 1911.

# EFFECTS OF AN ABORTIFACIENT PASTE ("UTRA-JEL")

## REPORT OF A DEATH FROM ITS USE AND OF AN EXPERIMENTAL STUDY OF ITS EFFECTS ON RABBITS AND RATS

R STRAUS, MD, AND N DE NOSAQUO, MD

LOS ANGELES

WASHINGTON, D C

The use of abortifacient pastes to produce abortion, as contrasted with other methods, is relatively uncommon. Except for an occasional editorial comment<sup>1</sup> and isolated reports,<sup>2</sup> information concerning the morbidity and the mortality resulting from the use of such pastes has not received much attention in the American and British medical literature. In the German literature extensive reports have appeared, concerned chiefly with deaths from peritonitis, intravascular hemolysis and air and fat embolism following the use of such pastes as "Interuptin," Leunbach's paste, "Interferin" and "Provokol."<sup>3</sup>

### REPORT OF A CASE

E M, a 22 year old white woman, about six weeks pregnant, was made to abort by a physician who introduced an abortifacient paste into the uterus twice at a two day interval. That an abortifacient paste was used, however, was not known until after the official investigation. The patient began to bleed from the vagina

This study was supported by the Zeiler Memorial Research Fund, aided in part by grants from Mr J Copeland and Mr B Oberstein.

From the Laboratories of the Cedars of Lebanon Hospital, Los Angeles (Dr Straus), and the Food and Drug Administration, Federal Security Agency Washington, D C (Dr De Nosaquo).

Dr G A Granger made the original investigations in this field for the Federal Security Agency in connection with the enforcement of the Federal Food, Drug and Cosmetic Act.

1 Abortifacient Pastes, report of Bureau of Investigation, J A M A 98 2155, 1932, Interferin, Queries and Minor Notes, *ibid* 105 1210, 1935, Abortion and Leunbach's Paste, editorial, *ibid* 111 535, 1938, Abortifacient Pastes, editorial, *ibid* 115 221, 1940, Abortionists Sentenced, Medical News (Minnesota), *ibid* 115 227, 1940.

2 Ridell, G A J Obst & Gynaec Brit Emp 39 1, 1932 D'Amour, F E, and Kiven, N Am J Obst & Gynec 29 503, 1935 Weilerstein, R W J A M A 125 205, 1944.

3 Wolfe, H Monatschr f Geburtsh u Gynak 88 442, 1931 Pankow and Pfeiderer Zentralbl f Gynak 56 865, 1932 Sellheim, Weischel, Kustner, Schweitzer and Vogt *ibid* 56 750, 1932 Engelman, F Deutsche med Wchnschr 58 166, 1932 Wagner, G A Monatschr f Geburtsh u Gynak 90 445, 1932 Engelman, F Zentralbl f Gynak 56 119, 1932 Sellheim, H Monatschr f Geburtsh u Gynak 90 441, 1932 Leunbach, J H *ibid* 90 446, 1932 Otto, K Zentralbl f Gynak 56 112, 1932 Brach, E *ibid* 56 122, 1932.

twenty-four hours after the second injection. The following day nausea, vomiting and generalized abdominal pain developed, which ultimately localized in the right lower quadrant of the abdomen.

She was admitted to a hospital on the fifth day of illness. The body temperature was 100.4 F. She appeared toxic and presented signs of peritonitis in the lower portion of the abdomen. She was treated with repeated blood transfusions and sulfathiazole for the infection, with morphine for the pain and with general supportive measures. Later pyuria developed, followed by urinary retention. Abdominal distention was also present. A mass gradually developed in the left lower quadrant of the abdomen. This suddenly decreased in size when an abscess ruptured spontaneously into the rectum on the forty-second day in the hospital. On the forty-fifth day in the hospital laparotomy revealed that the entire pelvis was filled with a hemorrhagic and purulent exudate. Her course in the hospital was downhill and was characterized by a high spiking temperature. Death occurred on the fifty-sixth day in the hospital.

**Autopsy**—Because of the homicidal aspects of the case, the body was brought to the coroner's office of Cuyahoga County in Ohio, where the autopsy was made by one of us (R S). The body was moderately emaciated. The incision in the abdomen was partially healed in the upper half and infected in the lower half. The labia were edematous. The gastrointestinal tract was distended, and loops of small intestine in the lower half of the abdomen were adherent. Between the loops were pockets of purulent and hemorrhagic exudate. Approximately 500 cc of firm blood clot and purulent exudate filled the pelvis. A perforation, 2 cm in diameter, passed from the cul-de-sac into the rectum.

The organs revealed no significant abnormality apart from the genitourinary system. The ureters were slightly constricted at their lower ends, owing to the local inflammation, they and the renal pelves were moderately dilated and hyperemic. The kidneys were slightly enlarged and showed severe ascending pyelonephritis.

The uterus was small but soft. The inner surface was greenish gray and irregular. The myometrium was similarly discolored and edematous. Near the right cornu of the fundus was a perforation 8 by 4 mm in diameter. The edges of the defect were rounded and greenish gray. The cervix was elongated, the external os showed healing lacerations, the tissues being discolored and edematous. Microscopically, the endometrium was extremely thin, regenerative in type, with moderate inflammation and areas of metaplasia to stratified squamous epithelium. Considerable hemosiderin was present, chiefly within endothelial phagocytes. The myometrium was edematous and moderately infiltrated with leukocytes. The sections from the area



of perforation revealed most extensive inflammation. In addition, embedded deep in the myometrium were irregular masses of orange-colored, somewhat granular material with a vacuolated and hyalinized background, resembling agglutinated masses of blood clot as though altered in part by chemical agents (fig 1). A few multinucleated foreign body giant cells were present

be seen a few small masses of vacuolated, deeply orange-stained material like that described in the uterus, with a similar foreign body giant cell reaction.

Smears and cultures of the peritoneal exudate gave a mixed flora with *Bacillus coli* predominating in the cultures. The postmortem blood culture from the heart produced a luxuriant growth of *Bacillus welchii*.

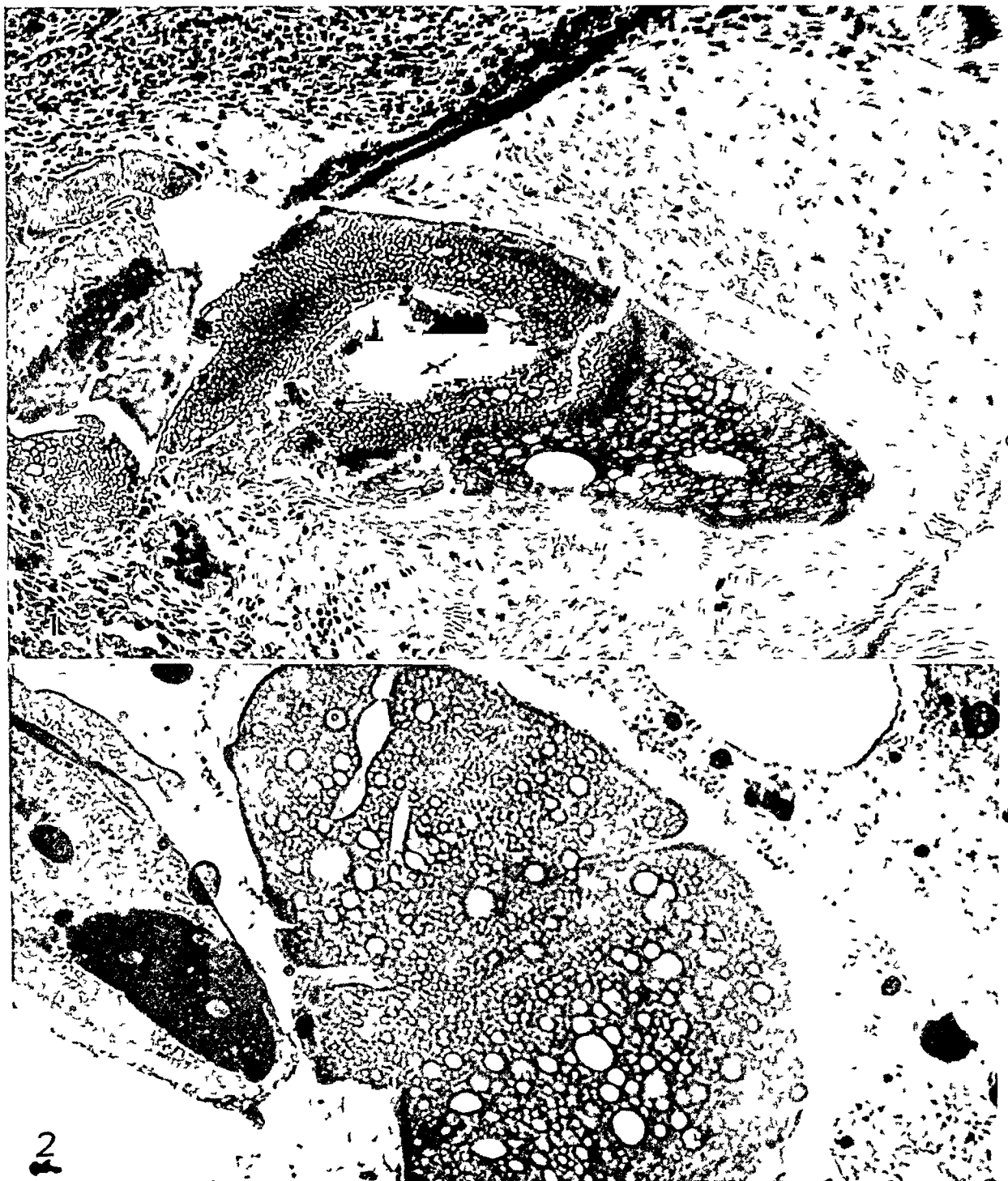


Fig 1—Lesion in a human uterus treated by injection of an abortifacient paste

Fig 2—Effect of the paste on human blood in vitro

near this material. The myometrium around it was diffusely necrotic, with only phantoms of the former structures. Organizing blood clot and purulent exudate in which were embedded small masses of the material just described covered the perimetrium.

The ovaries and tubes were buried in a mass of exudate. In one ovary were a number of infected follicular cysts and abscesses in some of which could

Because of the perforation in the uterus, the abortion at first was believed to be due to mechanical procedures, though a caustic chemical was suspected when on microscopic examination the orange-stained material with the regional foreign body reaction was seen. Official investigation revealed that an abortifacient paste had been used, and trial of the physician who injected the paste resulted in his conviction for manslaughter.

The results of a chemical analysis of the paste ("utra-jel") were reported as follows

Castor oil-potassium hydroxide soap	35.1 to 47.3	per cent
Pine oil	10.3 to 25.2	per cent
Alkali-combined iodine	1.1 to 1.6	per cent
Water	34.9 to 44.5	per cent
pH	8.1 to 9.49	

Since little is known of the effects of this paste, we decided to investigate its effects on human blood *in vitro* and in pregnant animals

#### ACUTE EXPERIMENTS WITH ABORTIFACIENT PASTE

*Effect on Human Blood in Vitro*—The abortifacient paste (0.5 cc) was added to fresh human blood (5 cc) in a test tube. This mixture was shaken vigorously for two minutes, allowed to stand for thirty minutes, then fixed with about five volumes of a neutral 4 per cent solution of formaldehyde for twenty-four hours. The solid portions of this mixture were then dehydrated in the usual procedure, embedded in paraffin, sectioned at 10 microns and stained with hematoxylin and eosin.

Almost immediately after the paste was added to the blood and shaken, the blood turned dark brown and became lumpy. The microscopic preparations of this material revealed a striking alteration of the blood clot (fig 2). The erythrocytes were no longer recognizable. Instead there were irregular masses of coarsely vacuolated material which was in part granular and in part hyaline. This tissue was deeply orange stained and presented a distinct similarity to the vacuolated masses found in the uterus and the ovary of the patient.

*Effect on Uterus of a Pregnant Rabbit*—One healthy female rabbit, weighing approximately 6 pounds (2.7 Kg), well along in pregnancy, was used. With the animal under anesthesia, approximately 0.5 cc of the abortifacient paste was injected in three places in the left horn of the uterus. One morning, a week later, the rabbit was found dead.

There was perforation of the uterus at one of the sites of injection. Generalized purulent peritonitis was present. No fetuses were found in either horn of the uterus. Portions of the tissues were fixed in a neutral 4 per cent solution of formaldehyde, dehydrated, sectioned and stained in the usual manner. Histologic examination revealed an extensive necrotizing inflammation of the left uterine horn at the sites of injection. In the wall at the site of perforation and in the lumen could be seen numerous small masses of vacuolated granular and hyaline material similar to those described in the human uterus and ovary. This material was not seen in the opposite horn of the uterus nor in sections of the heart, the lungs, the liver, the spleen and the kidney.

*Effect on the Uterus in Pregnant White Rats*—In 2 healthy pregnant white rats, each weighing about 200 Gm, approximately 0.5 cc of the abortifacient paste was injected into the vagina, and a cotton plug was inserted to prevent its escape. In 2 other pregnant rats, under anesthesia induced with ether, approximately 0.1 cc of the abortifacient paste was injected into the wall and the lumen of the right horn of the uterus in five places, each the site of a rat embryo. The left horn of the uterus was reserved as a control. Seven days later all the animals were killed, and the tissues were sectioned in the usual manner.

Histologic examination of the uterus revealed in each instance an extensive suppurative inflammation of the inner surface. In 1 rat an inflammatory stricture prevented the expulsion of two fetuses. All the others

had been aborted. In the lumen between the dead fetuses and the uterine wall could be seen small, irregular, vacuolated granular and hyaline masses of material, similar to those seen in the human uterus and ovary (fig 3). Both the uterine wall and the tissues of the embryos around these masses showed gangrenous necrosis as though acted on by a diffusible toxic agent. Sections of the heart, the lungs, the liver and the kidneys revealed no significant abnormality.

No alterations were noted in the tissues, including the vagina and the uterus, of those rats treated intra-vaginally with the paste.

#### CHRONIC EXPERIMENTS WITH THE ABORTIFACIENT PASTE

Five healthy pregnant white rats, each weighing about 200 Gm, were anesthetized, and approximately 0.1 cc of the paste was injected into the right horn of the pregnant uterus of each animal in from two to seven places. One animal died five days later. The remaining animals were killed forty-three days later. Tissues from all were sectioned and prepared in the usual manner.

Examination of the animal that died five days after being given injections revealed extensive generalized acute peritonitis secondary to a perforation of the uterus. The fetuses had been aborted. Histologic examination of the tissues showed extensive inflammation and characteristic vacuolated granular and hyaline masses similar to those in the acute experiment.

Examination of 2 of the remaining rats on the forty-third day after injection revealed no evidence of the fetuses. These animals showed no gross or histologic abnormality except for a diffuse eosinophil infiltration in the wall of the treated horn of the uterus. It is suspected that the paste was injected wholly into the lumen of the uterus in these animals and was then expelled. In the other 2 animals, inflammatory strictures caused the retention of two fetuses in one animal and only one fetus in the other. The fetuses were autolyzed. There was extensive chronic inflammation of the uterine wall with considerable regional fibrosis, chiefly in the region of the injection. Here, too, were numerous irregular masses of vacuolated granular and hyaline material, with regional foreign body giant cells and xanthoma cells (fig 4). This closely resembled the lesion in the human uterus and ovary.

#### EXPERIMENTS WITH THE COMPONENTS OF THE ABORTIFACIENT PASTE

*Ingredients Added to Human Blood in Vitro*—Approximately 0.5 cc amounts of castor oil, castor oil soap prepared with sodium hydroxide, oil of pine needles, 5 per cent sodium hydroxide and decolorized tincture of iodides were added, respectively, to 2 cc amounts of human blood in test tubes. The mixtures were shaken vigorously for about two minutes and allowed to stand for thirty minutes, then the solid portions were fixed, sectioned and stained in the usual manner.

Sodium hydroxide, castor oil and castor oil soap when added to the blood produced a brown discoloration immediately after being mixed, while the oil of pine needles and the tincture of iodides caused a delayed and less pronounced discoloration. Sections of the solid portions of these mixtures revealed a complete loss of cellular elements of the blood and only small irregular amorphous and granular masses of orange and brown material remaining in places. Nowhere did these resemble the lesion seen in figure 2.

*Ingredients Injected into the Uterus of White Rats*—In 5 nonpregnant healthy white rats, each weighing

about 200 Gm, in several places in one horn of the uterus was injected approximately 0.2 cc of castor oil, castor oil soap, oil of pine needles, decolorized tincture of iodides and 5 per cent sodium hydroxide, respectively. The animal given the injection of oil of pine needles died within thirty minutes. The animal given the injection of 5 per cent sodium hydroxide died on the

effect of this oil. Postmortem examination of the animal receiving sodium hydroxide revealed extensive local necrosis and inflammation but no characteristic lesion. The remaining animals, given injections of decolorized tincture of iodides, castor oil and castor oil soap, respectively, revealed only varying degrees of suppurative inflammation and focal necrosis.



Fig 3—Effect of the paste on the uterus of a pregnant white rat. Note a portion of a retained fetus on the right (seven days).

Fig 4—Effect of the paste on the uterus of a pregnant white rat (forty-three days).

second postoperative day. The other 3 rats were killed on the thirty-fourth day. The tissues of all the animals were prepared, sectioned and stained in the usual manner.

The animal given the injection of oil of pine needles revealed no characteristic changes either grossly or microscopically. Death was probably due to the toxic

#### COMMENT

The death of the patient was undoubtedly due primarily to the use of the abortifacient paste. Apparently the paste was sufficiently caustic to perforate the pregnant uterus and even erode

one ovary. The necrotizing action of the paste was demonstrated by the gangrenous necrosis of the uterine and fetal tissues in the rabbits and the rats, and the erosive action was apparent from the perforation of the uterus in 2 of the animals.

Characteristic of the effects of this paste are irregular, vacuolated, partially hyaline and partially granular masses, which are different from the lesions seen in other types of abortion or poisoning. As demonstrated by the in vitro experiments, characteristic masses are produced by the action of the paste on blood. The erythrocytes are laked and the proteins of the cells and the serum agglutinated into the granular and hyaline masses. The orange discoloration of these masses is due chiefly to the oil of pine needles and to some extent to the alkali. The vacuoles appear to be produced by enmeshed droplets of castor oil and oil of pine needles.

Once formed, these masses may remain in situ and induce granulomatous inflammation with foreign body giant cells. This result was well shown by the lesions in the human uterus and in the chronic experiments on white rats.

Since it was impossible to reproduce the lesion with any of the component parts of the

paste, either in vitro or in animals, this lesion is believed to be characteristic of the paste as a whole.

#### SUMMARY

A 22 year old white woman was caused to abort by intrauterine injections of an abortifacient paste. The paste appears to have been caustic and to have produced perforation of the uterus with generalized peritonitis and death. Masses of material believed to be the result of the action of the paste on blood were observed microscopically in the uterus, in the perimetrial exudate and in the ovary. Human blood mixed with the paste in vitro produced masses of material with microscopic characteristics similar to those in the human uterus. In a pregnant rabbit and in white rats the paste when injected into the uterus produced changes similar to those observed in the human uterus. The component parts of the paste when mixed one by one with human blood or when injected singly into the uterine wall in white rats failed to reproduce masses similar to those described.

The masses of material herein described are unlike any associated with other types of abortion and are probably characteristic of the type of abortifacient paste used.

# ADENOMYOEPITHELIOMA (CYLINDROMA) OF PALATAL MUCOUS GLANDS

WILLIAM H. BAUER, M.D., D.D.S., AND ROBERT A. FOX, M.D.  
ST. LOUIS

Billroth<sup>1</sup> coined the term "cylindroma" to designate a tumor which had arisen from the mucous glands of the accessory nasal sinuses and penetrated into the orbit. The tumor was characterized by a distinct parenchyma traversed by hyaline cylinders and tubes of stroma. In using the term "cylindroma" Billroth referred to the peculiar stroma rather than to the parenchyma. At first he regarded the cells of the parenchyma as epithelial, but a short time later he preferred to consider them as of connective tissue origin, precipitating a heated discussion as to the epithelial or the endothelial origin of the cylindroma. Although the endothelial concept seems to have been abandoned in favor of the epithelial one, there are still much confusion and lack of unanimity as to the genesis and the classification.

A large number of specimens of so-called cylindroma of the salivary and mucous glands have since been described as "mixed tumors." On the other hand, basically different neoplasms, such as true mixed tumors and adenoma, which occur in the region of the salivary glands and exhibit varying degrees of hyalinization, have been indiscriminately included in the cylindroma group. The occurrence of hyalinization in carcinoma with origin from salivary glands has led others to classify cylindroma as a variety of adenocarcinoma. The latter idea, propounded by McFarland<sup>2</sup> and by Mayo and Dockerty,<sup>3</sup> is supported by the "potential" malignancy of cylindroma. McFarland included cylindroma with the mixed tumors of the salivary glands, and stressed their generally unfavorable prognosis, particularly when subjected to surgical removal. He emphasized the "lack of correlation between the histopathology of the tumor and its clinical behavior" and expressed the belief that "the microscope, beyond showing that the lesion is a mixed tumor, is misleading." Mayo

and Dockerty, in their first paper on cylindroma, also classified it with the mixed tumors of the salivary glands. They stated that "the average grade of malignancy was higher in the 'mixed tumor' group" and on this basis differentiated between the two types of growth. In a later study, however, they reported two "metastasizing adenocarcinomas of cylindroma type." They regarded them as "pure cylindromas" and warned against confusing this highly infiltrative neoplasm with the more common mixed tumor, which has a vastly different prognosis. Other authors, however, have refused to accept cylindroma as definitely a cancer.

In this paper we do not presume to be able to solve the question of the nature of these tumors. We are fully aware of the limitations of microscopic evaluation in this respect. Our study was conducted with the following questions in mind:

1. How does the homogeneous stroma of cylindroma develop and what is its relationship to the parenchyma?

2. From what is the parenchyma of pure cylindroma derived?

3. From the histologic point of view can pure cylindroma of the palate be considered as a variety of mixed tumor or as adenocarcinoma?

## APPEARANCE OF THE TUMOR

The so-called cylindroma occurs wherever salivary or mucous glands are found. It forms slowly, is of elastic consistency and is usually freely movable beneath the mucosa or the skin. It tends to be sharply circumscribed. The cut surface of the tumor is grayish red and has a glossy, translucent, gelatinous appearance.

## HISTOLOGY OF THE PALATAL GLANDS

In the submucosa of the posterior portion of the hard palate and especially in the soft palate there are dense aggregations of tubuloalveolar mucous glands. These are, for the most part, connected to secretory ducts of varying diameter, and to isthmuses between the glands and the main secretory ducts. The epithelium of the isthmuses and main ducts is composed of cells of two types: cylindric cells and the so-called bas-

From the Departments of Pathology of the Schools of Dentistry and Medicine of St. Louis University.

1. Billroth, T. *Virchow's Arch. f. path. Anat.* **17** 357, 1859.

2. McFarland, J. *Surg., Gynec. & Obst.* **63** 457, 1936.

3. Mayo, C. W., and Dockerty, M. B. *Surg., Gynec. & Obst.* **74** 1033, 1942; *Surgery* **13** 416, 1943.

ket cells located between the cylindric cells and the basement membrane, as well as between the cylindric cells themselves. Detailed information regarding the structure of the basket cells can be found in an exhaustive study by Zimmermann<sup>4</sup>. In the parotid, submaxillary, sublingual and lacrimal glands the basket cells assume a stellate pattern because of radiating long threadlike extensions of the cytoplasm. In the mucotubular glands, however, they resemble fibroblasts because of their flat, broad spindle shape. Maximow and Bloom<sup>5</sup> and other investigators have considered these cells epithelial in origin and capable of contraction, a concept which induced Renaut<sup>6</sup> to call them "myoepithelial" cells. Their function is concerned with the expulsion of collected secretion within the glandular lumen. Because more force is required to extrude the sticky contents of the mucous glands as compared with the watery seroalbuminous contents of the albuminous glands, the former type contains far more basket cells than the latter. It is the basket cells, we believe, which play the important role in cylindroma of the mucous glands.

#### MATERIAL

Our histologic studies were confined to 3 tumors classified as cylindroma and 3 classified as adenocarcinoma of the palate. The specimens had been fixed in a 2 per cent solution of formaldehyde and embedded in celloidin (a concentrated preparation of pyroxylin). Detailed studies were limited to 1 particular specimen of cylindroma which was sectioned serially. This tumor of walnut size was singled out because it had been curetted two years prior to total excision. The curettage was done under the erroneous impression that the lesion was an abscess. In addition to the routine hematoxylin-eosin preparations, sections were studied with Mallory's aniline blue, Masson's trichrome and Best's congo red stain.

#### MICROSCOPIC FEATURES OF THE TUMORS STUDIED

The cytologic features of the tumors diagnosed as cylindroma varied considerably. Each of the growths was separated from the palatal epithelium by a remarkably thick, dense fibrous membrane (fig 1). Generally, there was negligible round cell infiltration in the subepithelial stroma, it was most marked in the previously curetted tumor. Here the epithelium of the mucosa grew deep into the neoplasm, gradually

infiltrating and intermingling with the parenchyma. The subepithelial fibrous membrane contained a moderate number of spindle-shaped cells and a few vessels with perivascular leukocytic infiltration. Sections stained with aniline blue according to Mallory's technic showed streaks of collagen fibrils arranged in whorls and strands, most of which were parallel to the epithelial surface. Radiating connective tissue septums divided portions of the tumor parenchyma into lobules (fig 2). Although the connective tissue which extended between the lobules was composed of a moderate number of spindle cells centrally, the peripheral portions assumed a more homogeneous appearance. In addition the connective tissue stroma had invaded the individual glandular or ductlike structures of the parenchyma (fig 2). It is especially noteworthy that the fibrous stroma had undergone hyaline degeneration wherever it was in contact with the secretion of the parenchymal cells (fig 3). In sections stained with hematoxylin and eosin a bluish product of the epithelial cells formed a sort of membrane of varying thickness around dense areas of epithelium and branched into the interior between groups of cells. The connective tissue hyalin was pink and appeared to be fused with the epithelial secretion, forming an epithelial-mesenchymal substance. It seemed as though the epithelial secretion incited the hyalinization of the fibrous stroma. This transformation of the fibrous stroma also occurred when it invaded the ductlike structures and came into contact with the epithelial secretion (fig 3). Special stains demonstrated the presence of elastin in the latter, but congo red failed to reveal amyloid, which Lubarsch<sup>7</sup> thought this homogeneous substance to be. There were areas entirely dominated by aggregations of this hyaline substance. The scanty remnants of degenerated cells within these areas furnished evidence that pressure had been exerted by the infiltrating stroma on the parenchyma. However, there was also evidence that the parenchyma had proliferated into the masses of hyaline substance. The larger homogeneous masses had a mottled pattern due to the presence of irregularly shaped lighter areas, the latter were the result of degeneration and disappearance of the tubular parenchyma (fig 4).

The bulk of each tumor formed a spongy mass due to anastomosing branches of tubular structures of varying caliber (fig 2). The tubules and the strands of cells could be traced to the normal mucous glands with which they were connected by what may be called transitional structures. It was these areas which clearly

4 Zimmermann, K. W. Die Speicheldrüsen der Mundhöhle und die Bauchspeicheldrüse, in von Mollendorff, W. Handbuch der mikroskopischen Anatomie des Menschen, Berlin, Julius Springer, 1927, vol. 5, pt. 1, pp. 61-244.

5 Maximow, A. A., and Bloom, W. A Textbook of Histology, Philadelphia W. B. Saunders Company, 1939.

6 Renaut, J. Compt. rend. Acad. d. sc. 89: 247, 1879.

7 Lubarsch, O. Virchows Arch. f. path. Anat. 122: 373, 1890.



Fig 1—Cylindroma of the palate. An area of the tumor is shown, separated from the normal palatal mucosa by a fibrous wall  $\times 100$

Fig 2—Extensions of the fibrous wall dividing the tumor into lobes. Note the hyaline substance between and within tubes lined by epithelial cells  $\times 200$

Fig 3—Intercalated ducts from a transitional area between normal mucous glands and neoplasm. Proliferation of myoepithelial cells and invasion of stroma are shown  $\times 500$

Fig 4—Accumulation of hyaline stroma with irregular light patches, a result of the disappearance of myoepithelial cells following atrophy due to pressure. Cell remains are scattered throughout the field  $\times 200$



showed that the parenchymal cells of the tumor originated from the palatal mucous glands or their intercalated ducts. Proliferation of the secretory as well as of the basket cells had repro-

tion were observed occasionally, which had flattened or even caused complete disintegration, due to pressure, of the tubular lining cells. The lumens of the ductlike structures were surrounded



Fig 5—Tubules reproducing intercalated ducts. The inner layer of epithelial cells assumes a secretory function. Note the proliferating myoepithelial cells.  $\times 500$

Fig 6—Adenomatous structure adjacent to the region of previous curettage. Note the pleomorphism and hyperchromatism of the lining cells.  $\times 480$

Fig 7—Strands of myoepithelial cells in a collagenous stroma which has become hyalinized. Some cells are undergoing pressure atrophy.  $\times 450$

duced tubules or acini of mucous glands fairly well, the cells, however, appeared to be somewhat hyperplastic and occasionally degenerated (fig 5). Excessive quantities of mucous secre-

and frequently obliterated by one or more layers of somewhat disfigured proliferating cylindric cells (fig 3). The peripheral as well as the central proliferating cells extended into the



stroma in solid cords to form pseudotubules filled with mucoid material, or cords of cells surrounding irregular spaces, some of which contained cellular debris

which manifested striking polymorphism and hyperchromatism without mitotic figures (fig 6) This tissue represented densely packed mucous gland isthmuses Their lumens were

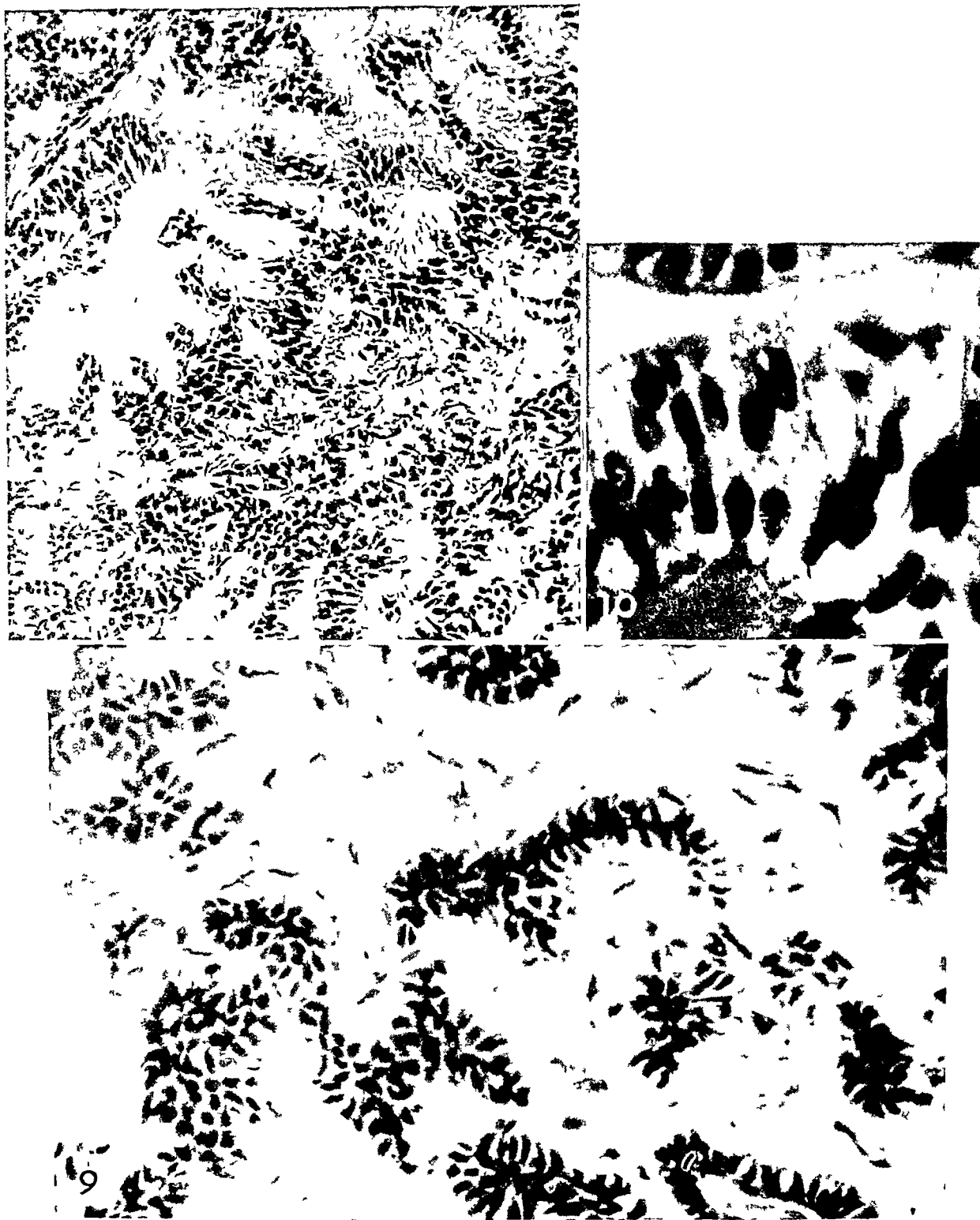


Fig 8—Hyalinization of fibrous stroma, proliferation of mucosal cells  $\times 400$

Fig 9—Network of tubules lined by a layer of flat epithelial and mucosal cells arranged like the needles of a pine tree  $\times 400$

Fig 10—Mucosal cells, the cytoplasm of which is fused with the hyaline stroma  $\times 600$

The secreting and the basket cells did not exhibit definite evidence of being cancerous. However, in the region of the former curettage there was a well defined adenomatous lobule

obliterated by either proliferating epithelial cells or mucus. Here and there the inner luminal lining cells principally, and the basket cells to a lesser extent, had undergone hyalinization and

keratinization Giant epithelial cells with large, hyperchromatic occasionally lobulated nuclei were found situated beside unusually small epithelial cells with pyknotic nuclei of varying degrees of chromatism The picture resembled the lesions produced by Fischer-Wasels<sup>8</sup> in his studies of the effects of carcinogens on the parotid glands of rabbits and rats

As stated before, the mass of each of our tumors consisted of strands or layers of proliferating myoepithelial cells (fig 7 and 8) One neoplasm was composed almost entirely of ducts lined by a single layer of flattened epithelium, while the spindle or ovoid peripheral cells were arranged like the needles of a pine tree (fig 9) Strands of similar spindle and ovoid cells were observed in another tumor, here they were separated by fibrous collagen which had been converted into a homogeneous hyaline substance in some places The nuclei of these cells were remarkably large, variously shaped and rich in chromatin There were no mitotic figures Some cells had cytoplasmic processes which extended in several directions, giving them a stellate appearance, and joined them with each other In other cells the cytoplasm was fused with the collagenous or hyaline stroma to such an extent that only the nucleus remained (fig 10)

#### COMMENT

It appears from the microscopic examination of our material that cylindroma originates from the palatal mucous glands, with which it is connected by transitory structures There was evidence of proliferation of the epithelial cells lining the intercalated ducts and especially of the basket or myoepithelial cells, located between the inner cylindric epithelial layer and the basement membrane The bulk of each tumor was composed of interlacing strands and cords of basket cells arranged in an adenomatous pattern The cytologic details cannot be fully understood unless one pays particular attention to the peculiar and intimate interrelation between the stroma and the parenchyma We feel that both the stroma and the parenchyma develop a proliferating, invasive capacity of remarkable strength However in this respect the evidence seems to indicate that the stroma is more powerful than the parenchyma, for it is the stroma which disrupts the epithelial aggregations and by pressure even produces atrophy The epithelium, however, may regain its infiltrative power and again invade the wide expanse of

stroma The fibrous stroma, which originates from the well developed capsule, meets in its advance the epithelial secretion, which causes it to undergo hyaline degeneration In those regions where there is no epithelial secretion the stroma maintains its fibrous character Observations and conclusions similar to these were arrived at by Herzog<sup>9</sup> in 1921

The secretion found in the ductlike structures of the tumors is the product of the cylindric lining cells Zimmermann<sup>4</sup> proved that the inner cylindric cells, as well as the cuboidal cells of the intercalated ducts of the palatal mucous glands, can be converted into large mucus-secreting cells If these lining cells diminish in size and their nuclei become smaller, their capacity to produce mucus vanishes

We have emphasized repeatedly the significance of the basket or myoepithelial cells in the structure of these tumors These cells and the odd relation between stroma and parenchyma, constitute the characteristic histologic feature We cannot share the opinion of those authors who consider the hyaline stroma alone the main diagnostic feature, because there are many epithelial growths which exhibit wide stromal hyalinization

Myoepithelial cells are found in the albuminous and mammary glands, as well as in the glands of Moll and the apocrine sweat glands They are not directly connected to the stromal tissue which surrounds the acini and intercalated ducts but are separated from it by a basement membrane, although they do not leave their imprint on this membrane, they do on the acinous and cylindric cells of the ducts As a rule, the cytoplasm contains fibrils which pass through the large dark nucleus However, we observed that wherever myoepithelial cells appear in close proximity to hyalinized stroma, the fibrillar cytoplasm also becomes hyaline, thus fusing itself with the stroma In sections stained with hematoxylin and eosin these cells are elongated and have large hyperchromatic nuclei The Masson stain, however, brings out a reddish cytoplasm, which helps to differentiate myoepithelial from secretory epithelial cells

The importance of the myoepithelial cell was recognized a long time ago Ribbert<sup>10</sup> and others demonstrated the ability which these cells have to proliferate after ligation of the excretory duct of the parotid gland of the rabbit or in inflammatory and regenerative processes involving that gland Some authors regarded them as

8 Fischer-Wasels, B Allgemeine Geschwulstlehre, in Bethe, A, von Bergmann, G, and others Handbuch der normalen und pathologischen Physiologie, Berlin, Julius Springer, 1927, vol 14, p 1477

9 Herzog, G Beitr z path Anat u z allg Path 69 422, 1921

10 Ribbert M W Sitzungb d med-rhein Gesellsch f Nat u Heilk 1879, p 86

germ cells of the secreting glandular epithelium, and Krompecher<sup>11</sup> called them "basal cells." In his studies of epithelioma of salivary and mucous glands Krompecher referred to a "reticular mucous cylindroma" which was composed of proliferated "basal cells" as a "basal cell adenoma." The tumors of the parotid gland described by French and German pathologists as acinous and canalicular adenoma must not be confused with Krompecher's "basal cell adenoma."

Sheldon<sup>12</sup> observed proliferation of myoepithelial cells in tumors of the sweat glands, and Kuzma<sup>13</sup> described proliferating elongated contractile cells in the human breast.

Recently, Sheldon<sup>14</sup> in an elaborate study of 54 tumors of the salivary glands divided his material into four groups. He regarded the tumors in the first group as adenoma derived from the secreting epithelium of the salivary glands. His second group was constituted of the "mixed tumors" because "in addition to the secreting epithelium, the basket cells, which are a normal component of the salivary glands, contribute not only to the tumor but are actually the predominating cells." He held that the normal picture is further distorted by "degeneration of the epithelium with subsequent myxomatous and pseudocartilaginous appearance of the stroma. In addition, squamous metaplasia of the epithelium may occur. True cartilage or bone which arises by metaplasia from the connective tissue stroma further confuses the picture." The tumors of the third group were characterized by basket cells alone without degenerative or metaplastic changes of the stroma. Sheldon suggested the name "myoepithelioma" for these slowly invading growths. The fourth group was composed of tumors diagnosed as carcinoma of the secretory epithelium.

The tumors classified as cylindroma of the palatal mucous glands which we examined do not fit into Sheldon's second group ("mixed tu-

mors") because of the absence of myxomatous, pseudocartilaginous tissue and squamous metaplasia, as well as of cartilage and bone. On the other hand, they cannot be placed in his third group because, though their bulk consists principally of myoepithelial cells, there are also adenomatous structures resembling normal mucous glands. The interlacing network of strands of proliferating myoepithelial cells in a hyalinized stroma of epithelial-mesenchymal origin associated with adenomatous formations of secreting cells induces us to suggest the term "adenomyoepithelioma" for these tumors of the palatal mucous glands. The absence of definite microscopic features of cancer delimits adenomyoepithelioma (cylindroma) from adenocarcinoma of the palate. However, we believe that though the growth is principally noncancerous, it may undergo transformation into adenocarcinoma. This alteration may develop spontaneously or may be encouraged by surgical intervention. However, if one wishes to retain the term "cylindroma," we should prefer to consider the cancerous growth as carcinomatous cylindroma rather than as cylindromatous carcinoma.

#### SUMMARY

Cylindroma of the palate arises from the cells lining the intercalated ducts of the palatal mucous glands. Not the cylindric cells but the basket or myoepithelial cells chiefly form the bulk of the parenchyma of this tumor. There is an intimate relationship between the erstwhile cylindric cells which differentiate into secreting mucous cells and the fibrous connective tissue stroma which radiates from the well preserved capsule into the parenchyma. We believe that the fibrous stroma invades the tubular epithelial formations and by coming into contact with the secretion of the latter becomes converted into a hyaline substance.

Although this tumor of the mucous glands may be remotely related to the "mixed tumors" we interpret it as a specific type of neoplasm. The name "adenomyoepithelioma of the palatal mucous glands" seems to be appropriate. The tumor though genuinely benign may undergo cancerous alteration.

11 Krompecher, E. Beitr. z. path. Anat. u. z. allg. Path. **70** 489, 1922.

12 Sheldon, W. H. Arch. Path. **31** 326, 1941.

13 Kuzma, J. F. Am. J. Path. **19** 473, 1943.

14 Sheldon, W. H. Arch. Path. **35** 1, 1943.

# EXPERIMENTAL NEPHROPATHIES

## I A METHOD OF PRODUCING CONTROLLED SELECTIVE INJURY OF RENAL UNITS BY MEANS OF CHEMICAL AGENTS

JAMES P SIMONDS, M.D., AND OPAL E HEPLER M.D.  
CHICAGO

In his comprehensive review of experimental nephropathies in 1937, Horn<sup>1</sup> remarked that "the abnormalities reported following the use of chemical agents and irradiation have but a remote application to the human disease

The value of such procedures resides more significantly in the pursuit of functional and toxicologic studies" Most of the contributors to the extensive literature on the effects of chemical agents on the kidneys have not differentiated between these two points of view Usually, investigators have been more concerned with the certainty of damaging the kidneys than with producing a selective effect by the use of the poison The dose employed was not, therefore, adapted to the requirements of an accurate study of renal function

One of the chief difficulties that has confronted the experimental pathologist in this field has been the lack of dependable means of identifying the particular portion of the renal tubule damaged by a chemical agent Suzuki<sup>2</sup> and Aschoff and Suzuki<sup>3</sup> used the vital dye phenol red to differentiate the three parts of the proximal convoluted tubule Edwards<sup>4</sup> macerated portions of kidneys injured with mercury bichloride, dissected them with glass needles under a microscope and towed the isolated tubules to an area of the dish used in dissection, where it was uncoiled as far as possible in order to identify the different segments Both of these methods are tedious, time consuming and difficult In the course of our studies during the past seven years on the effects of chemical agents on the kidneys of dogs we have developed a method by which the first and the terminal

portion of the canine renal tubule can be selectively injured with considerable accuracy and later identified in microscopic sections with reasonable certainty

### DESCRIPTION OF METHOD

Each dog was weighed and its total blood volume was determined approximately by the formula  $\text{weight in grams} \times 0.0925 = \text{blood volume in cubic centimeters}$  Then a carefully measured quantity of the poison in isotonic solution of sodium chloride was slowly injected intravenously The amount administered ranged from 0.10 to 3.0 mg per hundred cubic centimeters of blood At proper intervals, usually from eighteen to seventy-two hours, the animals were killed and their kidneys studied microscopically with the aid of several staining methods

In our experiments potassium dichromate, mercury bichloride and uranyl nitrate were used as renal poisons Suzuki<sup>2</sup> was one of the first to present evidence, based on more accurate methods of differentiating the parts of the proximal convoluted tubule, that the dichromate affects the first part, while the bichloride and uranyl nitrate injure particularly the terminal portion This claim has been frequently disputed Recently, Edwards<sup>4</sup> insisted that the bichloride does not cause necrosis of the terminal portion of the proximal convoluted tubule He used rabbits, guinea pigs, rats and frogs in his experiments The following comments on the work of Edwards are pertinent In the first place, he teased out an individual tubule from macerated renal tissue and identified the affected portion of the tubule by its thinness For purposes of identification and description he divided each tubule into eight (presumably equal) segments In the second place, the poison was administered intraperitoneally (except in the frogs), and the total dose per kilogram of body weight was much higher than that used in our experiments—ranging in the rabbits from 2.44 to 5.88 mg per kilogram Furthermore, Edwards found a significant species difference in the distribution of the necrosis in the kidneys of the

From the Department of Pathology, Northwestern University Medical School

This investigation was aided by a grant from the Committee on Therapeutic Research of the American Medical Association

1 Horn, H. Arch Path **23** 71 and 131, 1937

2 Suzuki, T. Morphologie der Nierensekretion unter pathologischen Bedingungen, Jena, G. Fischer, 1912

3 Aschoff, L., and Suzuki, T. Verhandl d deutsch path Gesellsch **15** 199, 1912

4 Edwards, J. G. Am J Path **18** 1011, 1942



Figures 1, 2 and 3

## EXPLANATION OF FIGURES 1, 2 AND 3

Fig 1—Renal tissue of dogs poisoned with potassium dichromate ( $K_2Cr_2O_7$ ) (a) Three doses of 1 mg each of the poison per hundred cubic centimeters of blood were given to dog K Cr 1 on three successive days. The animal was killed on the fourth day after the first dose. Necrosis is seen to be limited to the subcapsular zone and to the labyrinth, none is present in the straight terminal portions of the proximal convoluted tubules  $\times 55$  (b) Four milligrams of the poison per hundred cubic centimeters of blood was given to dog K Cr 8. The animal was killed on the third day. Marked necrosis may be seen in the subcapsular zone and labyrinth. Some necrosis is present in the straight portions of the proximal convoluted tubules  $\times 55$

Fig 2—Renal tissue of dogs poisoned with mercury bichloride ( $HgCl_2$ ) (a) One dose of 3 mg of the poison per hundred cubic centimeters of blood was given to dog K Hg 8. The animal was killed on the fourth day. No necrosis is present in the subcapsular zone. Marked necrosis may be seen in the straight terminal portions of the proximal tubules near the center of the photomicrograph  $\times 55$  (b) The same dose of this poison was given to dog K Hg 6. The animal was killed on the third day. Necrosis is more extensive than in dog K Hg 8. This illustrates the individual differences in susceptibility of different dogs when fairly large doses are administered  $\times 55$

Fig 3—Renal tissue of dogs poisoned with uranyl nitrate ( $UO_2[NO_3]_2$ ) Two doses of 1 mg each of this poison per hundred cubic centimeters of blood were given to dog K Ur 1. The animal was killed on the third day. Necrosis is not present in the subcapsular zone or in the labyrinth. Necrotic straight terminal portions of the convoluted tubules are seen in the lower part of center  $\times 55$  (b) Three doses of 1 mg each of the same poison per hundred cubic centimeters of blood were given to dog K Ur 2 on successive days. The animal was killed on the fourth day. Slight necrosis is seen in the subcapsular zone and labyrinth, marked necrosis, in the straight terminal portion of the proximal convoluted tubules in the lower part of the photomicrograph  $\times 55$

mammals used by him. In the rat the necrosis was most frequently observed in the fifth and sixth segments, in the rabbit, in the sixth and seventh segments, while in the guinea pig it extended from the eighth segment upward for varying distances, even to the point of junction of the tubule with the glomerulus. The animals used by Edwards survived for periods ranging from less than one day to one hundred and thirty-four days.

It is of particular interest that, as demonstrated by Edwards, the location of the maximal injurious action of mercury bichloride in the proximal convoluted tubules varies in different species of animals. Our experiments were limited entirely to dogs. When minimum necrotizing doses of the chemical agents are injected intravenously into dogs, potassium dichromate causes necrosis of the first part, and mercury bichloride and uranyl nitrate of the terminal portion, of the proximal convoluted tubule. This can be demonstrated in dogs by two quite simple devices.

1 In the kidneys of dogs and other mammals there is a narrow zone immediately beneath the renal capsule which is devoid of glomeruli. This zone is composed almost entirely of the first portions of proximal convoluted tubules which come off from the more superficial, or peripherally located, glomeruli, with a few distal convoluted tubules. In the kidneys of dogs poisoned with the smallest necrotizing dose of potassium dichromate, necrosis was present in this narrow subcapsular zone (fig. 1 a). In the deeper portions of the cortex, the necrosis was in the labyrinth and involved transverse or sharply curved short sections of tubules, which were probably segments of the first portions of the proximal convoluted tubules of glomeruli situated more deeply in the cortex. The straight, terminal portions of these tubules, which lie chiefly in the outer part of the labyrinth along the margins of the medullary rays, were not necrotic when the dose was properly selected. Larger but still very small doses caused more extensive necrosis (fig. 1 b). On the other hand, in the kidneys of dogs poisoned with the minimum necrotizing dose of mercury bichloride or of uranyl nitrate, necrosis was not seen in the narrow subcapsular zone but was present in the straight terminal tubules deep in the cortex along the margins of the labyrinth (figs. 2 a and 3 a). With equivalent doses, the necrosis caused by mercury bichloride seemed to extend farther upward into the proximal convoluted tubules than did that caused by uranyl nitrate. A possible reason for this difference will be discussed in a later paragraph. Slightly larger doses of these substances also caused more widespread necrosis (figs. 2 b and 3 b).

2 In sections of the kidneys of dogs stained with sudan III we repeatedly noted the presence of fat in tubules that lay in the zone of junction between the labyrinth and the medullary rays (fig. 4). The transition from fat-free to fat-containing epithelium in a tubule may be quite abrupt. Later we found that Modell<sup>5a</sup> had made a similar observation and had stained isolated tubules dissected out of macerated kidneys of dogs and cats. He found by this means that the fat was present in the straight, terminal portion of the proximal convoluted tubule of the dog and in higher segments in the kidneys of cats. Modell's observations were confirmed and corrected by Foote<sup>5b</sup>. It seemed, therefore, that fat in this location in normal canine kidneys might be used to identify the lower or terminal portions of the proximal convoluted tubules. We studied sudan-stained sections of the kidneys of 163 dogs in order to determine what

*Presence of Fat in Terminal Portions of Proximal Convoluted Renal Tubules of Dogs Given Various Poisons*

	Fat Not Sufficient for Identification per Cent	Fat Sufficient for Identification, per Cent	Number of Dogs
Snake venom	75.0	25.0	20
Streptococcus toxin	50.0	50.0	6
Diphtheria toxin	47.8	52.2	23
Normal controls	45.2	54.8	42
Staphylococcus toxin	41.7	58.3	12
Mercury bichloride	23.5	76.5	17
Potassium dichromate	14.4	85.6	21
Uranyl nitrate	9.1	90.9	22
			163
Classified summary of results			
Glomerular poisons	55.7	44.3	61
Normal controls	45.2	54.8	42
Tubular poisons	15.0	85.0	60
			163

percentage of them contained fat. This group contained normal controls and dogs that had been poisoned with various substances. The results were surprising. For purposes of classification these kidneys were divided into two groups: (a) those in which the epithelium of the terminal portions of the proximal tubules contained sufficient fat for satisfactory identification and (b) those in which fat was either not present or present in such small amount that it could not serve the purpose of identification of parts of the tubules.

The results are shown in the accompanying table. The classified summary at the end of the table is of particular interest. More than half of the normal control dogs had sufficient fat in the terminal portions of their proximal convoluted tubules to permit identification of these

5 (a) Modell, W. Anat. Rec. 59:253, 1934. (b) Foote, J. J. Proc. Soc. Exper. Biol. & Med. 34:196, 1936.



structures. Recognition of the fact that a specific part of the tubule of the normal canine kidney may contain fat in appreciable and even in considerable amount may aid in avoiding an incorrect interpretation of the results of experimental procedures on these organs. However, the type of poison used does appear to have a definite effect on the quantity of fat found in the tubular epithelium.

The venom of snakes (moccasin and rattlesnake) and the toxins produced by streptococcus, staphylococcus and the diphtheria bacillus are all proteins and therefore colloids, although their

Mercury bichloride, potassium dichromate and uanyl nitrate are crystalline substances and therefore pass easily through the glomerular filter. By absorption of water from the glomerular filtrate, they are concentrated in the tubules. Since their most evident effect is on the tubular epithelium they have been classed as tubular poisons in the table. Eighty-five per cent of the kidneys of this group of dogs contained sufficient fat to identify the straight terminal portions of the proximal convoluted tubules. The difference between this group and the normal controls (about 30 per cent) is statistically

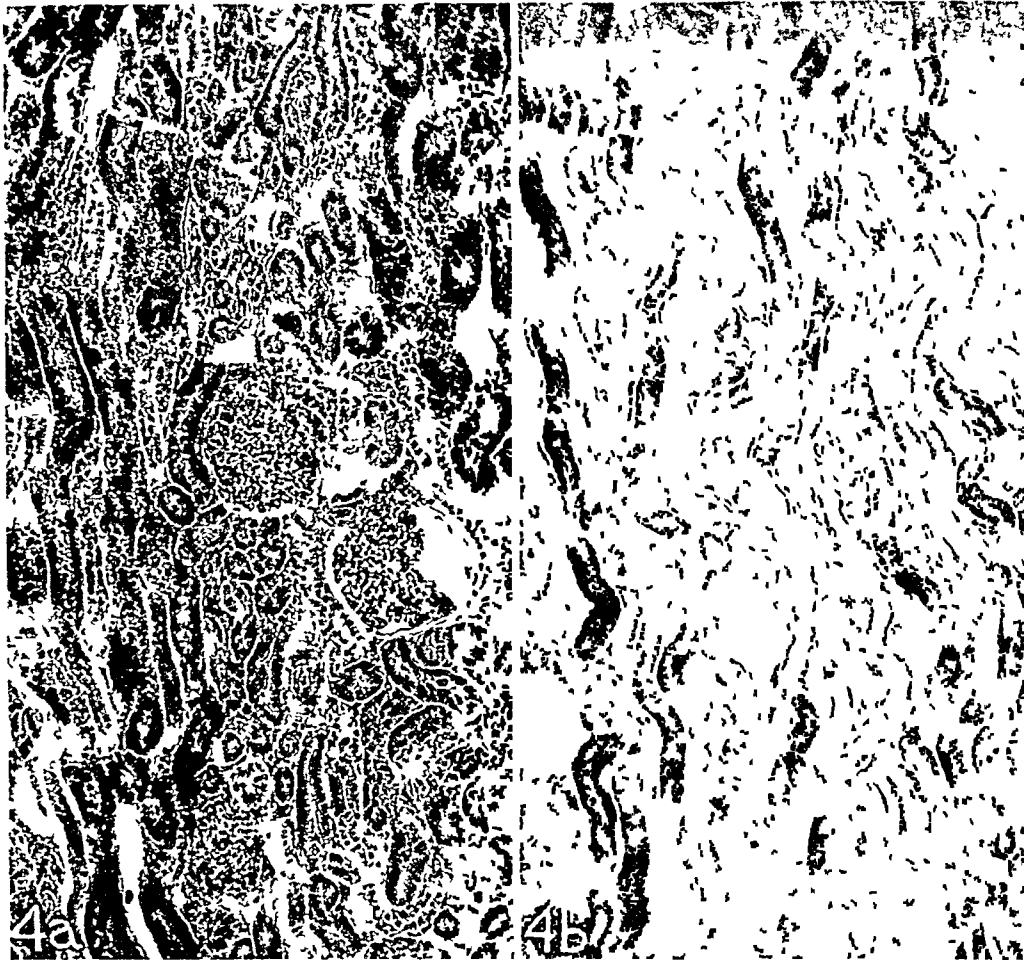


Fig 4—Renal tissue of dogs poisoned with mercury bichloride ( $\text{HgCl}_2$ ) and potassium dichromate ( $\text{K}_2\text{Cr}_2\text{O}_7$ ) (a) One milligram of mercury bichloride per hundred cubic centimeters of blood was given to dog K Hg 3. The animal was killed on the fourth day. Sudan III stain was used. Fat, reproduced as black droplets, is seen in the straight terminal portions of the proximal convoluted tubules. (b) One dose of 4 mg of potassium dichromate was given to dog K Cr 8. Sudan III stain was used. Many of the cells containing fat are necrotic.

molecules are small in comparison with those of some of the proteins. Colloids do not pass readily through the glomerular filter and tend to become concentrated in the capillaries because of the loss of water by filtration. It is for this reason that these substances have been classed as glomerular poisons. It will be noted that the kidneys of the dogs to which these poisons were administered contained somewhat less fat than did those of the normal controls. The difference (about 10 per cent) may not be sufficient to be significant.

significant. It appears, therefore, that these tubular poisons increase the tendency of the specific tubular epithelium of dogs to accumulate fat and thus aid in identification of the part of the tubule involved.

These two methods of identifying the first and the terminal portions of the proximal convoluted tubules can be applied only if the minimum necrotizing dose of the poison is administered. When in a dog after the injection of a properly selected dose the necrosis of tubular epithelium is found to be limited to the sub-



capsular, aglomerular zone and to the labyrinth, leaving the straight, fat-containing, terminal portion without visible damage, that poison may be said to affect the first segment of the proximal convoluted tubule. When, on the other hand, after such an injection the necrosis is found to be limited to the straight, fat-containing, terminal portion, leaving the subcapsular zone intact, that poison may be said to have affected the terminal segment of the proximal tubule.

Aside from possible differences in relative toxicity, an explanation of the involvement of different parts of the proximal convoluted tubule may be related to a fact pointed out by one of us in another connection.<sup>6</sup> Presumably it is the metal ions in the molecules of each of the poisons that injure the renal epithelium. It is assumed also that such a metallic poison in weak solution can damage the cells only by entering them and that the amount entering the cells is proportional to the concentration of the poison in the tubular lumen. The concentrations of the metallic ions in each of the three poisons used in these experiments differ among themselves. One milligram of potassium dichromate contains  $6.80 \times 10^{-3}$  gram atoms of chromium, 1 mg of mercury bichloride contains  $3.66 \times 10^{-3}$  gram atoms of mercury, and 1 mg of uranyl nitrate contains  $2.53 \times 10^{-3}$  gram atoms of uranium. If the absorption of water proceeds uniformly throughout the proximal convoluted tubules, a concentration of chromium ions capable of injuring the renal epithelium would be attained at a higher level in the tubules than would the concentration of metallic ions with a corresponding dose of either of the two other poisons. Potassium dichromate would therefore be expected to damage a portion of the proximal convoluted tubules nearer to the glomerulus than would either mercury bichloride or uranyl nitrate.

Perhaps a more accurate comparison of the toxic effects of these poisons could be made if the amount of each substance injected was proportional to the gram atom equivalent of the respective metal in the molecule than if merely an equal number of milligrams was used, as was done in these experiments. This would certainly be necessary if one had to determine the relative toxicity of the three substances.

Except for minor variations due to individual differences in susceptibility to one or another

of the poisons used in these experiments, the degree of injury of the tubular epithelium varies with the quantity of poison administered. For this special reason and for more general reasons microscopic examination of the kidneys of treated animals is essential to a correct interpretation of the results of experiments on renal function based on the use of such agents. More than 3 mg per hundred cubic centimeters of blood of any of the substances used in these experiments will produce necrosis throughout the greater portion of the proximal convoluted tubule. With the smallest necrotizing dose—usually 1.5 to 2 mg per hundred cubic centimeters of blood—potassium dichromate causes necrosis that is sharply localized in the first part of the proximal convoluted tubule, and mercury bichloride and uranyl nitrate, in the terminal portion. With smaller subnecrotizing doses, visible damage—swelling and splitting of the epithelium—can be detected in that portion of the tubule in which the particular poison characteristically produces its necrotizing effect. The conclusion would seem to be justified, therefore, that an extremely small dose, incapable of producing visible injury, would induce disturbances of function that would be limited even more closely to that part of the tubule in which the particular poison in larger doses produces its characteristic necrosis.

This method of controlled selective injury of specific parts of the nephrons by properly chosen doses of chemical substances, adequately checked by microscopic examination of the kidneys, lends itself to more accurate studies of the functional activities of these organs than methods heretofore employed. It should be of value in determining the mechanisms of the clearance of substances, such as urea and dextrose, that are absorbed by the renal tubules, and of substances, such as diodrast and exogenous creatinine, that are excreted by these structures. We have used this controlled method in studies of the clearance of urea, endogenous creatinine, inulin and diodrast in dogs. The results will be published in another paper.

#### SUMMARY

Controlled selective injury of specific parts of the renal units may be produced by means of chemical agents and the part of the proximal convoluted tubule affected identified. This may be applied in the study of renal function.

# Case Reports

## MYELOMALACIA OF THE CERVICAL PORTION OF THE SPINAL CORD, PROBABLY THE RESULT OF ROENTGEN THERAPY

LEWIS D STEVENSON, M D, AND ROBERT E ECKHARDT, M D, P H D, NEW YORK

The side effects of roentgen therapy are principally (a) direct injury to the cells as evidenced by necrosis, vacuolation of the cytoplasm and abnormal mitoses, (b) vascular changes, characterized by endothelial thickening and proliferation, with resultant thrombosis, and degeneration of the elastica and the muscle cells of the media; (c) hyalinization of the collagen.<sup>1</sup> These changes occur in all tissues, including those of the central nervous system. However, it is the vascular change which seems to offer the most serious complication of radiation therapy directed to the central nervous system.<sup>2</sup> It has been our experience to observe a man believed to have a metastasis of lymphoepithe-

area to the left of the midline. The Kline test was negative. Biopsy of this lesion revealed lymphoepithelioma. Roentgen therapy was given through five portals as outlined in the accompanying table. This first course of therapy was given from Oct 21, 1941 to Jan 2, 1942.

This exposure was followed by complete disappearance of the lesions. The patient complained only of dryness of the pharynx and the nasal passages, an inevitable result of the radiations he had received. In November 1942 a necrotic area developed in the midline of the posterior part of the hard palate, which responded to local irrigations.

He was examined at approximately three month intervals thereafter. On Sept 8, 1943, a 2 cm node was found lying in the soft tissues over the insertion of the right sternocleidomastoid muscle. To this node he received 4,500 r (15 × 300 r) with 200 kilovolts, 15 milliamperes, at 50 cm target skin distance and with a

### Roentgen Treatments

Portal	Location	Size, Cm	Treatments	Factors			Filters, Mm	Total r
				Kilo volts	Milli amperes	Target Skin Distance, Cm		
A	Intraoral	2.5 around	10 × 400 r	200	15	40	0.5 Cu + 1 Al	4,000
B	Right temporal	6 around	16 × 250 r	200	15	50	1 Cu + 1 Al	4,000
C	Left temporal	6 around	16 × 250 r	200	15	50	1 Cu + 1 Al	4,000
D	Right cervical	13 × 6	16 × 250 r	200	15	50	1 Cu + 1 Al	4,000
E	Left cervical	12 × 9	23 × 250 r	200	15	50	1 Cu + 1 Al	5,750

lioma of the nasopharynx after intensive roentgen therapy to the neck and the oropharynx. Postmortem examination revealed not the expected metastasis but myelomalacia probably secondary to vascular changes from the radiation therapy.

### REPORT OF A CASE

F K, a 43 year old Turkish waiter, when first seen in the New York Hospital Clinic, on Sept 29, 1941, complained that he had noted a painless swelling of the left side of the neck for three months. He gave a history of having had syphilis for fifteen years, previously treated for four months by injections made in the hip and the arm. On the left side was a firm, nontender, freely movable postauricular node, 3 by 2 by 2 cm, and on the right some smaller (0.5 cm) firm, nontender freely movable posterior cervical nodes. Careful examination of the nasopharynx, with the patient under local anesthesia, revealed a 1.5 cm grayish-covered

filter of 0.5 mm of copper and 1 mm of aluminum. The node disappeared, and an ulcer of the skin over the site healed promptly. In the early part of November he began to notice stiffness in the left arm and the left leg, and when this progressed to the point where he was no longer able to carry on his work, he was admitted to the hospital, December 29. On admission he said that he had first noted jumping of the left leg while riding in the subway train or sitting with his legs crossed. This was followed by stiffness and clumsiness of the left arm and the left leg. He became constipated and found that on three occasions he had urinated involuntarily. His penis felt dead, and he had become impotent. General physical examination disclosed nothing remarkable except for induration of the tissues of the neck in the region of the previous radiation therapy. Roentgen examination of the chest revealed only apical pleural thickening and mild emphysema. Neurologic examination revealed hyperreflexia of the left side, left finger stretch, absence of abdominal reflexes of the left side and a Babinski sign on that side. There was marked motor weakness of the left side, not including the face. These signs were interpreted as indicating disease of the left corticospinal tract below the head.

Reexamination three days later showed that a finger stretch and a Babinski sign had appeared on the right side, that sensation to pinprick was lost below the third thoracic segment bilaterally, that sensation of heat was

From the departments of pathology and medicine of the New York Hospital and the Cornell University Medical College

1 Warren, S. *Physiol Rev* 24:225, 1944

2 Warren, S. *Arch Path* 35:127, 1943

lost below the fourth cervical segment bilaterally but that sensation of touch was normal throughout. Lumbar puncture showed clear spinal fluid under an initial pressure of 100 mm of water. Manometric findings were normal. The protein was 42 mg per hundred cubic centimeters, the cell count, 0, the colloidal gold curve, 1122111100, and the Wassermann reaction of the spinal fluid, negative. The urine and the blood count were unremarkable, and the Mazzini flocculation test of the blood for syphilis was negative. An electroencephalogram showed a normal record, class II-I.

It was felt that these abnormalities might rise from a rapidly expanding intramedullary lesion in the region of the fourth cervical segment, possibly a metastasis

(11 × 300 r), with 200 kilovolts, 15 milliamperes at a 50 cm target skin distance and with a filter of 0.5 mm of copper and 1 mm of aluminum, was given.

Despite the therapy the patient's condition became progressively worse. He had increasing difficulty clearing mucus from his throat, a left-sided headache developed, and there was evidence of paralysis of the intercostal muscles with signs of atelectasis and bronchopneumonia in both lungs. On Feb 24, 1944 he died, apparently of respiratory paralysis, oxygen and morphine being of no avail.

*Autopsy*—There was 100 cc of serous fluid in each pleural cavity. There were scattered areas of con-



Fig 1—Section of the cervical region of the spinal cord stained for myelin. It shows extensive demyelination and areas of necrosis.

Cervical laminectomy was therefore performed and the cervical part of the cord explored on Jan 5, 1944. The cord and the dura from the sixth cervical vertebra to the foramen magnum appeared normal, with no evidence of epidural or intramedullary growth and no apparent vascular changes.

Following operation there was no improvement in this man's condition; rather the signs and symptoms progressed to include both sides, and complete urinary incontinence developed. Since it was thought that the laminectomy had ruled out late radiation fibrosis and adhesive arachnoiditis of the cord, a course of roentgen therapy was directed to the cervical cord for the possible effect on any undisclosed metastasis. Through an 8 cm portal in the posterior cervical region 3,300 r

solidation in all lobes, particularly in the upper lobe and both lower lobes of the right lung. There were large areas of fibrous pleural thickening over both upper lobes.

An increased amount of cerebrospinal fluid was noted in the meninges.

The bony trabeculae of the vertebrae were slightly softer than usual. The marrow was gray-red.

*Microscopic Observations*—The lungs showed patchy areas of consolidation with alveolar edema fluid and cells which were mainly polymorphonuclear.

No tumor cells were seen in the nasopharynx. Tissue from the region of the laminectomy revealed atrophy of muscle fibers and proliferation of muscle sheath cells, with formation of mononuclear giant cells and

ductlike structures that in places simulated a true gland. No tumor cells were seen. Several sections including bone and soft tissue showed profound injury compatible with that produced by roentgen rays, with atrophy of bone marrow and foci of necrosis, and perivascular infiltrations, but no thrombosis of vessels.

The vertebral marrow was infiltrated in large areas by epithelial cells with collections of lymphocytes, conforming with the diagnosis of lymphoepithelioma.

Sections through the cervical region of the spinal cord, stained for myelin sheaths, showed extensive loss of myelin in the anterolateral columns of both sides, somewhat more marked on one side than on the other. There was also considerable loss of myelin in the

changes. There was little reactive gliosis in the demyelinated areas or elsewhere. Sections of the cord in the thoracic and lumbar regions showed no such vascular changes but merely wallerian degeneration of the descending pathways—chiefly of the pyramidal tract.

#### COMMENT

Lyman, Kupalov and Scholz<sup>3</sup> demonstrated experimentally in dogs that roentgen rays have a profound effect on the central nervous system. It was their conclusion that this effect is not due to a primary effect on the neurons, but

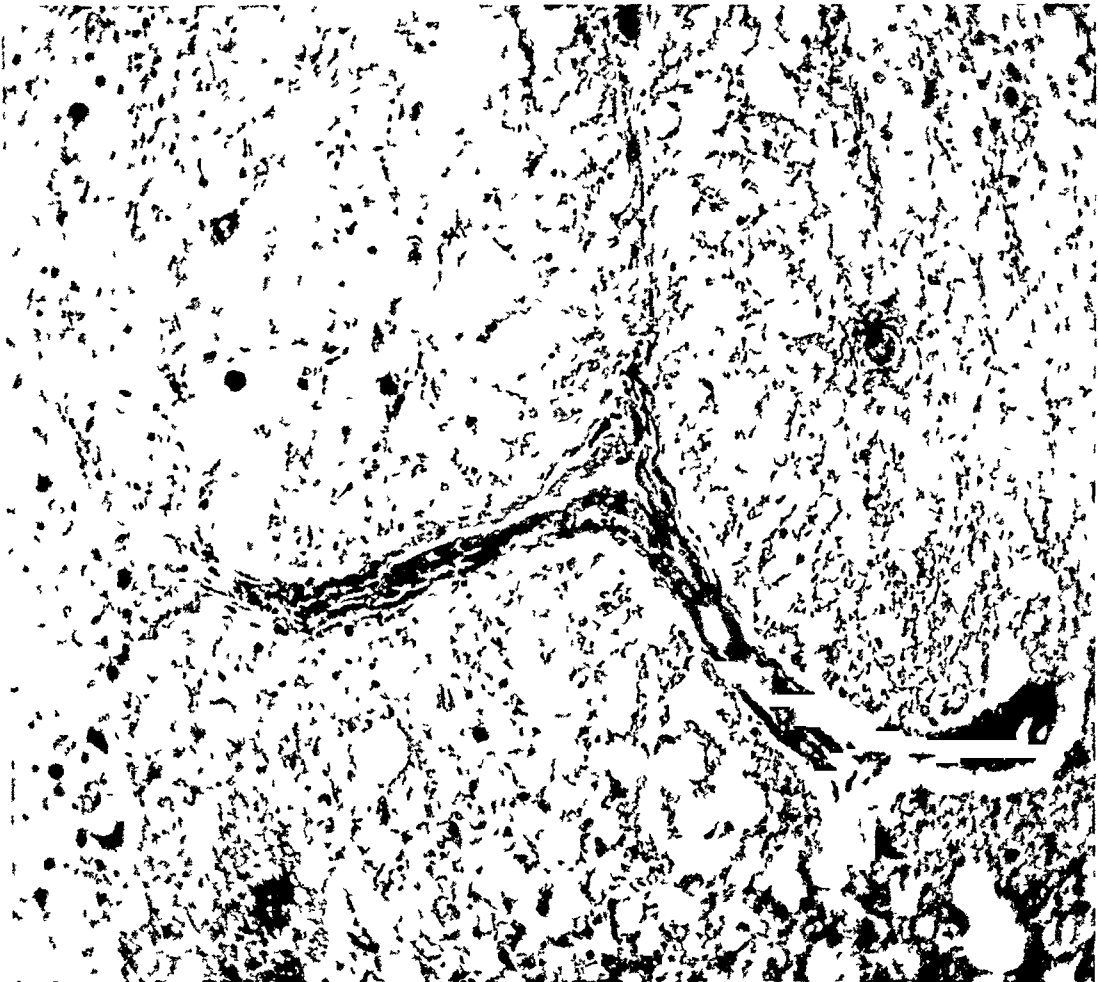


Fig 2—Small artery in the white matter with a greatly thickened fibrous wall and a narrowed lumen, surrounded by demyelinated areas. A few lymphocytes can be seen in the outer portion of the arterial wall.

posterior columns of the spinal cord. In the demyelinated areas a number of small necrotic foci were present (fig 1). The meninges were normal. Sections of the same region stained by the trichrome method showed extensive changes in the blood vessels of the white matter and the gray matter. These changes for the most part were fibrous thickening of the smaller arteries and arterioles with considerable narrowing of the lumens of these vessels (fig 2). About some of the vessels, a few lymphocytes were present in the perivascular spaces, and an occasional mononuclear cell was present in the adventitial coat of the artery. Sections stained with hematoxylin and sudan III showed the demyelinated areas to be densely packed with cells containing fat. There was an excessive amount of fat in many of the cells of the anterior horns. The cells of the anterior horns were reduced in number, and some of them showed vacuolation and other degenerative

results from changes secondary to a profound vascular reaction consisting of hyaline degeneration and obliterating sclerosis of arterioles. Colwell and Russ<sup>4</sup> and Stafford Warren (Duggar<sup>5</sup>) likewise reached the same conclusion. Alpers and Pancoast,<sup>6</sup> however, did not observe thick-

3 Lyman, R. S., Kupalov, P. S., and Scholz, W. *Arch Neurol & Psychiat* 29:56, 1933.

4 Colwell, H. A., and Russ, S. *X-Ray and Radium Injuries: Prevention and Treatment*, London, Oxford University Press, 1934.

5 Duggar, B. M. *Biological Effects of Radiation*, New York, McGraw-Hill Book Company, Inc., 1936.

6 Alpers, B. J., and Pancoast, H. K. *Am J Cancer* 17:7, 1933.

ening of vessels in human brain tissue surrounding tumors which had been subjected to radiation. Smithers, Clarkson and Strong<sup>7</sup> described a case in which a lesion of the spinal cord developed one year and three months after the patient received therapy for a carcinoma of the upper third of the esophagus. A Brown-Sequard syndrome resulted from a unilateral lesion in the left side of the spinal cord at the level of the third and fourth thoracic segments. The lesion was intramedullary, and there was no sign of block in the spinal subarachnoid canal. It was postulated that the lesion could only be one of intramedullary gliosis, i. e., spinal gliosis, which may or may not be an early stage of syringomyelia. The cerebrospinal fluid was normal and the Wassermann reaction negative. The level of the lesion was in the previously irradiated region, and it was calculated that the spinal cord at this point received a dose of 5,800 r in thirty-nine days. No postmortem observations were available, since the patient was still living.

We are presenting our case because we believe that the lesion observed represents an unusual end result of radiation therapy not previously reported in the literature. The fact that metastases of the lymphoepithelioma were found in the vertebral bone marrow at autopsy is sufficient indication that intensive radiation therapy was necessary. When depth-dosage factors are

taken into consideration, it may be calculated that our patient received from 6,000 to 8,000 r at the level of his spinal cord. It was the opinion at the time of admission that this amount of radiation could not have been responsible for the clinical appearance of this man on admission and the progress of his disease after admission. The fact that postmortem microscopic examination revealed degenerative changes of the spinal cord closely associated with thickened arteriolar walls warrants the conclusion that the degenerative changes were secondarily the result of the radiation therapy.

#### SUMMARY

A man received intensive radiation therapy in the cervical region for lymphoepithelioma of the nasopharynx. About two years later signs of transverse myelitis developed in the region of the fourth cervical segment. This myelitis progressed and eventually led to death from respiratory paralysis. At laminectomy the cord appeared normal. Further radiation therapy was without effect on the course of the disease. Postmortem examination revealed myelomalacia of the cervical part of the cord, in the vicinity of which many thickened arterioles with fibrous walls could be seen. This is believed to be an unusual reaction to roentgen ray therapy directed to the neck, but one which must be kept in mind when such therapy is contemplated.

<sup>7</sup> Smithers, D. W., Clarkson, J. R., and Strong, J. A. *Am J Roentgenol* **49**: 606, 1943.

## MEDIASTINAL TERATOMA

LIEUTENANT COLONEL SLOAN J WILSON AND MAJOR REUBEN CARES  
MEDICAL CORPS, ARMY OF THE UNITED STATES

Dermoid and teratomatous tumors of the mediastinum are relatively rare. Hertzler<sup>1</sup> in 1916 reviewed the literature as far back as 1825 and was able to find only 72 reported cases. Hedblom<sup>2</sup> in 1933 added 113 cases. Phenister, Steen and Volderauer<sup>3</sup> in a later survey to and including July 1936 stated that reports of 208 cases had been published, of which only 48 were cases of teratoma. Houghton<sup>4</sup> reported an instance of true teratoma of the mediastinum and found only 24 cases in the literature that he would accept as being cases of cancerous mediastinal or intrathoracic teratoma.

The literature from 1930 to the present date (June 1944) has been reviewed, and several other cases should be added to the aforementioned surveys. The following investigators have reported single cases of teratoma arising from the three germ layers: Hablutzel<sup>5</sup>, de Castro and Parreira<sup>6</sup>, Moir<sup>7</sup>, Stanbury and Oille<sup>8</sup>, Wheatley<sup>9</sup>, Cooper and Henry<sup>10</sup>, Doran and Lester<sup>11</sup>, Becker<sup>12</sup>, van Joost and Kopp,<sup>13</sup> and Aguilar<sup>14</sup>. Two cases have been described by Cabot<sup>15</sup>. Harrington<sup>16</sup> reported 6 cases in which the tissue elements had arisen from all three germ layers. One of these cases is included in the review of Houghton<sup>4</sup>.

The accounts of several unusual instances of teratoma have been published. Struthers<sup>17</sup> reported a case of teratoma of the thymic gland in an infant 5 months of age. The thymic duct was still present. Fox and Haspers<sup>18</sup> described 2 unusual teratomatous tumors of the anterior mediastinum, one from two germ layers at least, and the other containing tissue of both liposarcoma and rhabdomyosarcoma. Schenck<sup>19</sup> reported a congenital teratoma of the aorta in a stillborn infant. Somolinos<sup>20</sup> published an account of a teratoma of the pericardium arising from all three germ layers.

It is the purpose of this publication to report a case of cancerous teratoma of the mediastinum arising from all three germ layers.

### REPORT OF A CASE

An enlisted man, white, aged 29, was admitted to a station hospital June 7, 1943, with a complaint of a hard, dry cough. The onset of the cough was about three weeks prior to admission. He had been coughing mostly at night, and the prone position seemed to aggravate and induce paroxysms. The patient had reported on sick call about three days after his symptoms were first noted. At that time a swelling of his neck was discovered. Shortly thereafter his collars were tight. The swelling in the neck, according to the patient, had not been progressive.

Examination revealed the skin to be smooth and elastic with a few lesions of acne on the posterior aspect of the chest. The face was florid. The neck revealed a mass in the left anterior cervical triangle, measuring about 10 by 6 cm, extending down sub-sternally and also filling the suprasternal notch. The mass was soft, rubbery, without tenderness and movable. The trachea and the larynx were displaced 3 to 4 cm to the right. The thyroid gland appeared to be buried beneath the mass. The veins of the neck were engorged. The veins of the left upper region of the chest and the left arm were widely dilated. The patient's temperature throughout his hospitalization ranged between 99 and 100 F.

A roentgenogram of the chest revealed a dense homogeneous mass occupying the entire upper mediastinum and extending into the neck. Its lower border was at the level of the ninth thoracic vertebra. It extended widely into the midlung fields bilaterally, and its outline was fairly smooth. The trachea was displaced to the

- 1 Hertzler, A E. *Am J M Sc* **152** 165, 1916
- 2 Hedblom, C A. *J Thoracic Surg* **3** 22, 1933
- 3 Phenister, D B, Steen, W B and Volderauer, J C. *Am J Roentgenol* **36** 14, 1936
- 4 Houghton, J D. *Am J Path* **12** 349, 1936
- 5 Hablutzel, C. *Schweiz med Wchnschr* **63** 1308, 1933
- 6 de Castro, J R, and Parreira H. *An de med int* **3** 935, 1934
- 7 Moir, P J. *Brit M J* **1** 463, 1936
- 8 Stanbury, W S, and Oille, W A. *J Tech Methods* **16** 52, 1936
- 9 Wheatley, G M. *Am J Dis Child* **54** 1057, 1937
- 10 Cooper, D A, and Henry, C M. *Internat Clin* **2** 253, 1939
- 11 Doran, W T, and Lester, C W. *J Thoracic Surg* **8** 309, 1939
- 12 Becker, B J P. *South African M J* **13** 659, 1939
- 13 van Joost, C R N F, and Kopp, J G. *Geneesk tijdschr v Nederl-Indie* **81** 969, 1941
- 14 Aguilar, H D. *Publ d centro de invest fisiol* **4** 309, 1940
- 15 Cabot, R C. *New England J Med*, **211** 689, 1934 **224** 207, 1941
- 16 Harrington, S W. *J Thoracic Surg* **7** 191, 1937

- 17 Struthers, R R. *Canad M A J* **26** 68, 1932
- 18 Fox, J P, and Haspers, C A. *Am J Cancer* **28** 273, 1936
- 19 Schenck, B. *M Rec* **144** 276, 1936
- 20 Somolinos, G. *Arch cardiol y hemat* **17** 152, 1936

right and compressed. Densities were present in the right cardiophrenic angle, which were interpreted as a pneumonic process or as atelectasis due to mucus.

On June 10, a lymph node in the left side of the neck was obtained for biopsy. The node was almost completely replaced by tumor tissue with the exception of a hemorrhagic pole seen grossly. Microscopically, this area contained a number of residual lymph follicles separated by proliferating trabeculae. Most of the normal nodal tissue had been replaced by neoplastic cells. These were arranged in sheets and broad cords of neoplastic cells measuring from 30 to 60 microns in diameter. The nuclei were highly vesicular, with a delicate nuclear membrane and a single or double stellate or spheroid nucleolus. The cytoplasm was moderately

sternum and in the left axillary and the left scapular region. Treatment with roentgen rays of high voltage was given over a period of seven weeks, totaling 32 exposures with a total of 3,525 r being delivered to the skin. During the early part of the therapy the mass regressed 2 cm. It then maintained its size. A new nodule appeared in the sternal notch, which became progressively larger. A short course of treatment with an unfiltered mixture of erysipelas toxin and *Bacillus prodigiosus* cultures (Coley's toxin) was given without any apparent beneficial result. Because of the failure to elicit further regression in the size of the tumor and because of the patient's desire to be nearer home, arrangements were made for transferring him to another general hospital. En route, the patient and

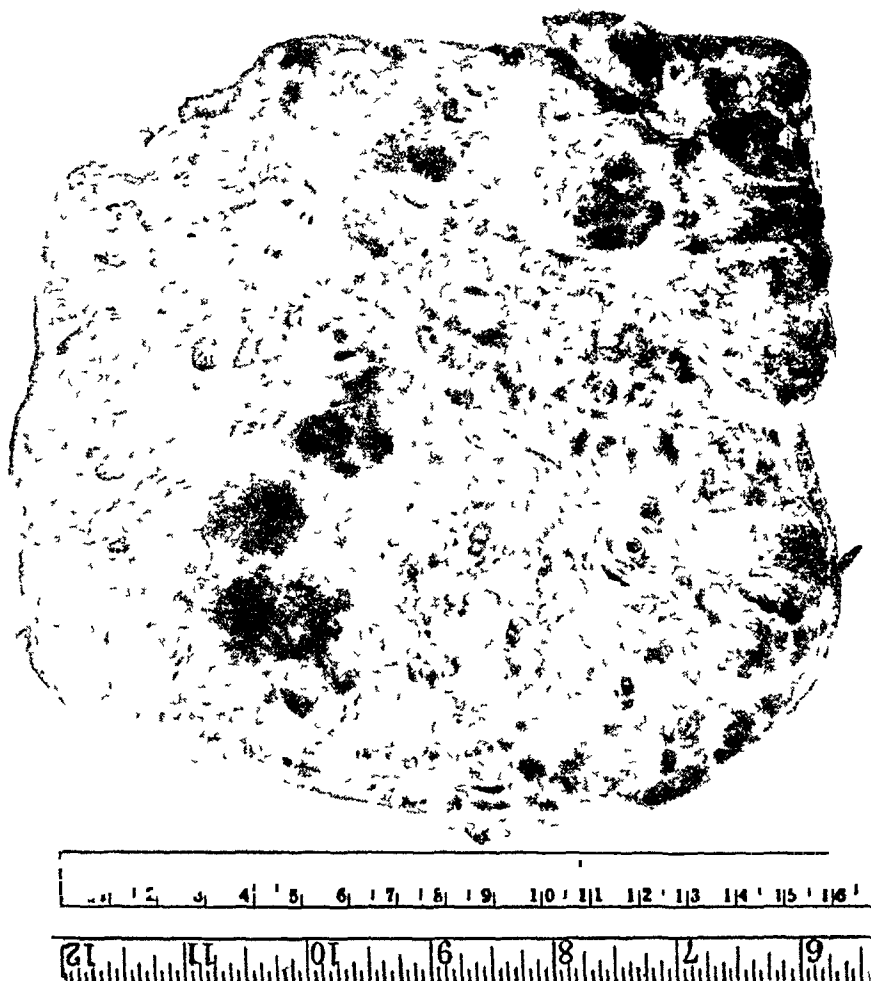


Fig 1—Photograph of the gross specimen showing the small cystic areas in many portions of the teratoma.

abundant, pale staining, granular and foamy, with occasional areas of distinct cell outlines. Frequent mitotic figures were present throughout, both regular and atypical forms. A number of tripolar figures were present. A diagnosis was made of lymphadenoma of the reticulum cell type. Biopsy specimens were forwarded to a histopathologic center. The opinion of the pathologist was that the neoplasm in the cervical lymph node was metastatic and was possibly representative of a metastatic teratoma of the testis. Slides were then forwarded to the curator of the Army Medical Museum, and the following report was received: "We believe that the tumor is metastatic in the cervical lymph node. It is too undifferentiated to permit speculation as to the primary site."

The patient was transferred to a general hospital. There were dilated veins over the upper portion of the

his attendant missed a train connection. He was admitted to a general hospital in the city to rest and walked into the admitting room. Shortly after admission the patient became dyspneic and was given oxygen. No particular significance was attached to this phenomenon, as the patient's attendant stated that this had happened on several occasions. On this occasion, however, the patient became progressively worse, and death ensued.

**Autopsy**—The neck was markedly enlarged, particularly in the left supraclavicular region. The neck measured 17 cm in width. The axillary and inguinal lymph nodes were palpable but small. The liver extended 7.5 cm below the xiphoid process. There was about 300 cc of clear straw-colored fluid in each pleural cavity. The superior mediastinum was wide and filled with a hard tumor mass. The mass extended down



over the pericardium and through the superior aperture of the thoracic cage into the lower portion of the neck, particularly on the left side. The pericardial sac contained about 200 cc of clear straw-colored fluid. On removal the tumor mass measured 18.5 cm in width, 17.5 cm in its superior-inferior aspect and 9.6 cm in thickness. It completely surrounded the ascending por-

tion of the aorta and part of the arch. The trachea was not involved. The tracheal lymph nodes were only slightly enlarged. On cut section the tumor mass contained strands of what appeared to be fibrous tissue. Small cystic areas were scattered throughout. The tumor was hard, and alternate areas of pink and yellow were noted. Areas of hemorrhage were also present.

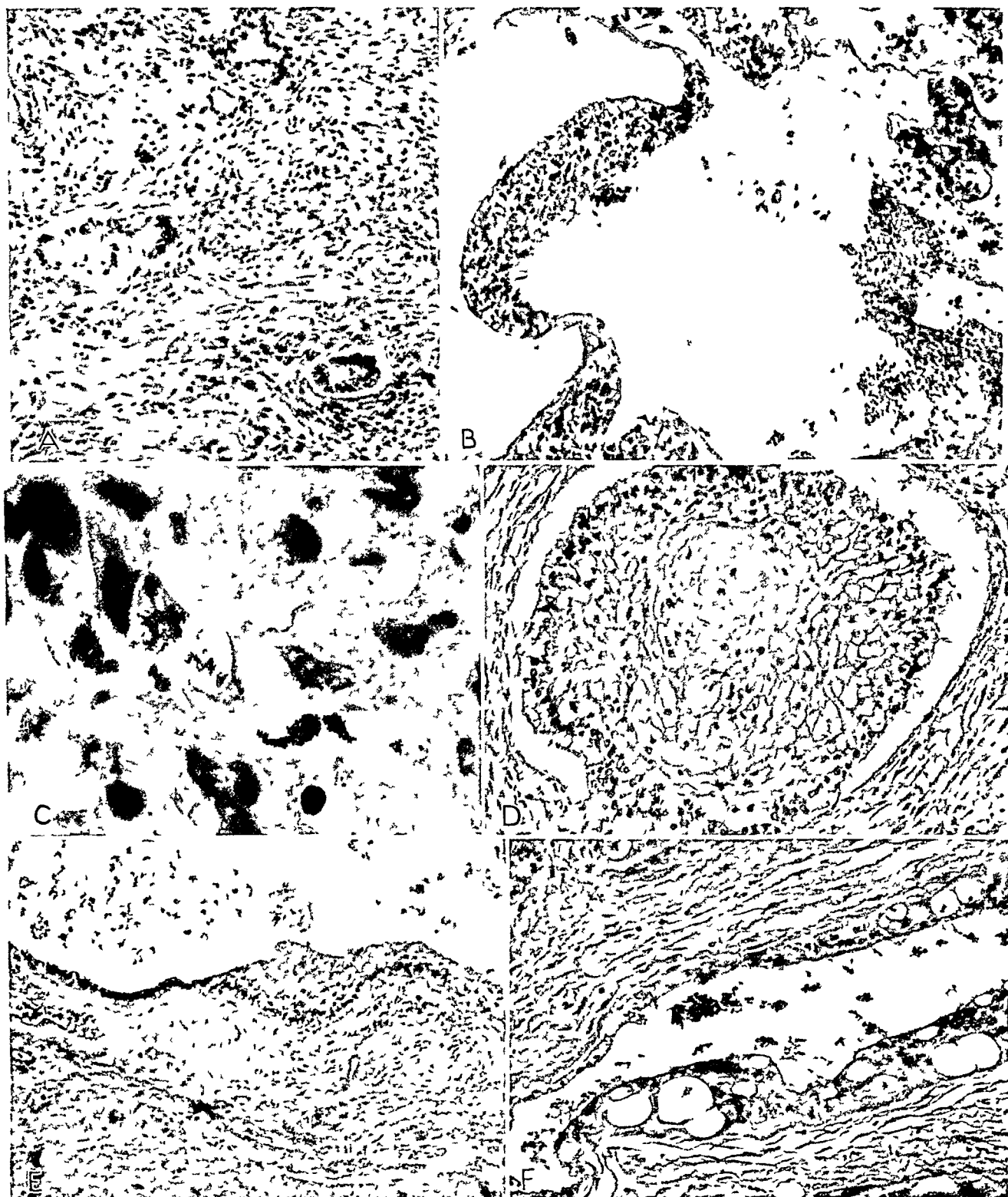


Fig 2—Photomicrographs of various tissue elements in the mediastinal teratoma. *A*, *B*, and *C*, choriocarcinomatous elements. *A*, tissue resembling endometrium with cytotrophic stroma and glands in various stages of preservation  $\times 100$ . *B*, tissue resembling choriocarcinoma. The "villus" structure contains clear cells of the Langhans type  $\times 100$ . *C*, high power view of cells in *B*. Note the syncytial cells and others with paler cytoplasm and vesicular nuclei  $\times 400$ . *D*, *E* and *F*, other epithelial structures. *D*, clear cell pseudo pearl formation. Many solid clear cell nests scattered throughout the tumor resembled closely adrenal cortical tissue  $\times 120$ . *E*, metaplastic transition of clear cell epithelium such as that in *D* from a simple columnar type  $\times 90$ . *F*, hydropic degeneration in quasisquamous cyst lining  $\times 150$ .



The tumor showed no spread into either pleural cavity by direct extension. The neck was thoroughly dissected. The thyroid gland was not involved in the neoplastic process. No discrete lymph nodes were found. The entire tumor mass in the neck appeared to be an extension of the mediastinal neoplasm. The trachea was markedly compressed where it passed through the superior aperture. The lungs were thoroughly dissected, and no neoplasm was found. The testes did not contain any tumor masses.

*Microscopic Examination*—The tumor was surrounded by a fibrous capsule. The matrix of the tumor was composed principally of myxomatous tissue and loose fibrous connective tissue. Scattered throughout almost every section examined were cysts lined with many different types of epithelium. Some cysts were lined with cuboidal epithelium, with an eosin-staining homogeneous material in the lumens. Other cysts contained little or no material in their lumens. The epithelial structures ranged from the simple columnar to the pseudostratified columnar type. In several of the sections the cysts were lined with stratified squamous epithelium. The lumens contained desquamated keratinized material, marked parakeratosis was also present. Cross sections of blood and lymph vessels were scattered throughout almost every section. In a few slides there were cells comparable to the cortical cells of the adrenal gland. Small areas of necrosis were scattered throughout. No extensive areas of hemorrhage were seen in any section examined. Tissue was present resembling endometrium with cytogenic stroma and glands in various stages of preservation. Other areas resembled choriocarcinoma.

#### COMMENT

There are many and varied classifications of teratoma of the mediastinum. Hedblom<sup>2</sup> described three types of teratoma: (1) epidermoid cyst containing only derivatives of ectoderm, (2) a dermoid type from ectoderm and mesoderm and (3) the true teratoma from all three embryonic layers. Askanazy<sup>21</sup> divided the true teratoma into two types: (1) the adult or cutaneous type and (2) the embryonal type, solid, of young growing tissues, with great inherent powers of proliferation. Harrington<sup>10</sup> observed that many mediastinal tumors may appear to be of the simple dermoid type, yet if careful sections of the wall are made, various tissues may be found which have arisen from all three germ layers. On this basis he objected to the classification of true teratoma as being a more or less solid tumor arising from all three germ layers and prefers to group all such tumors under the term "teratoma" and then qualify them according to the type of tissue which predominates.

The teratoma herein reported is the embryonal type, solid, and composed of young growing tissues in which there are cellular elements of a chorionomatous type. At first glance the cellular structure of the metastatic lesion in the cervical lymph node appeared to be entirely different from the main tumor mass of the mediastinum.

The metastatic lesion apparently was a proliferation from a single germ layer. In many respects it had the appearance of a metastasis from a seminoma of the testis, and this possibility was suggested by one of the pathologists reviewing the case. Figure 2B illustrates a villus which when examined under high magnification (fig. 2C) contained large pale cells of the Langhans type associated with masses suggestive of the syncytium noted in the metastatic lesion. The large pale glycogen-rich cells in the lymph node also resembled the chorionic cells of Langhans.

Numerous hypotheses have been formulated to explain the relationship of teratoma, particularly that of the testis and the ovary, and the appearance of choriocarcinomatous elements. Schlagenhauser<sup>22</sup> advanced the theory that every choriocarcinoma arises from an embryonal teratomatoid element in which there is cancerous proliferation of an isolated blastomatous element with proliferation of fetal chorion. Ewing<sup>23</sup> recorded a number of testicular tumors giving rise to chorioma, in which several limited areas of syncytial cells were present, the origin of which was not clear. Hemorrhage is not necessarily a constant feature of choriocarcinoma. With such a tumor hemorrhages may be absent or negligible in multiple pulmonary metastases.

Little mention is made in the literature of chorionomatous elements in mediastinal teratoma. Arendt<sup>24</sup> reported a case of gynecomastia in a 20 year old man with a mediastinal teratoma showing choriocarcinomatous transformation. Houghton<sup>4</sup> was of the opinion that there could be a hormonal stimulus of a quiescent teratoma of the mediastinum from hyperplasia of the normal interstitial testicular cells, such a stimulus inciting unrestricted neoplasia of reproductive chorionic elements. Becker<sup>12</sup> described elements resembling choriocarcinoma in the pulmonary metastases of a mediastinal teratoma. Chorionic villous structures were present in the case of Doan and Lester<sup>11</sup> and were intermingled with various tridermal structures. According to Budde,<sup>25</sup> the totipotential cells of the blastomeres are dissociated before the development of separate germ layers in those cases in which teratoma gives rise to choriocarcinoma.

#### SUMMARY

A solid teratoma of the mediastinum arose from all three germ layers in a white man aged 29 years. The teratoma contained choriocarcinomatous elements and had metastasized to a lymph node in the posterior triangle of the neck.

22 Schlagenhauser, cited by Ewing<sup>23</sup>

23 Ewing, J. *Neoplastic Diseases*, Philadelphia, W. B. Saunders Company, 1941.

24 Arendt, J. *Fortschr. a. d. Geb. d. Röntgenstrahlen* 43: 728, 1931.

25 Budde, M. *Beitr. z. path. Anat. u. z. allg. Path.* 68: 512, 1921.

21 Askanazy, M., cited by Houghton<sup>4</sup>

# General Reviews

## ARTERIOSCLEROSIS

W C HUEPER, M D

NEW YORK

## THE ANOXEMIA THEORY

*(Continued from Page 65)*

### COLLOIDAL PLASMATIC DISTURBANCES

#### A CARBOHYDRATIC TYPE

The anoxemic arteriosclerogenic mechanism active in colloidal plasmatic disturbances differs fundamentally from that present in the vasotonic and the hydrostatic hypotensive and hypertensive disorders. The interference with vascular oxygenation here is not caused by stagnation or ischemia resulting from abnormalities in the speed of blood flow, the amount of blood supply and the pressure exerted by the vascular content on the vascular walls but is due to an impairment of the exchange of gases and other nutritive material through the interface between the blood and the vascular wall by the interposition of a film or a precipitate formed by some normally colloiddally dispersed component of the plasma and causing subsequently a reduction in the permeability of the vascular walls. Such a disturbance in the colloidal equilibrium and stability of the plasma may be brought about by derangements in the quantitative relations or the physicochemical status of the normal colloidal constituents of the plasma (proteins, carbohydrates, lipoids) or by the appearance of abnormal endogenous colloids in the plasma (hypermolecular and hypomacromolecular serum proteins) or by the introduction of exogenous colloidal agents into the blood for therapeutic or experimental purposes (gelatin, ovalbumin, azoproteins, chemoallergic antibodies and antigens, heterogenic glycogen, polyvinyl alcohol, methyl cellulose, pectin, acacia [Hueper]). After injuring the endothelial lining through interference with its metabolic activity, these agents penetrate with the plasma into the intima and finally also into the media of the vascular wall and become an integral part of its structure by being deposited in the interstices and by being taken up into the cytoplasm of endothelial cells and histiocytes. In adjudging the morphologic appearance of the vascular changes elicited by these substances consideration must be given

to the fact that colloidal lipoids and carbohydrates when taken up into the proteinic cytoplasmic matrix form a heterogenous emulsion giving rise to a foamy cytoplasmic structure. The inclusion of proteinic matter in the cells, on the other hand, results in the production of a homogenous emulsoid.

The concepts just developed presuppose that molecularly or micellarily dispersed colloidal substances are taken up by the endothelial cells of the vascular wall and penetrate into the vascular wall. The normal artery is permeable for water, salt solution, dextrose, bile pigments and hemoglobin. This permeability serves nutritional purposes. The degree of permeability fluctuates with the width of the vessel, the blood pressure and the temperature (Zettler, Lange and Donomal, Lange and Sebastian). Many vasoactive drugs, such as sodium nitrite, theobromine, sodium salicylate, theophylline, theophylline ethylenediamine, mersalyl and others, produce a definite and rapid increase in vascular permeability, while other drugs of this type, such as calcium chloride, calcium gluconate and nicotine, depress the permeability of the vascular wall.

Perfusion experiments on isolated pieces of arteries, as well as in vivo studies, have shown that highly dispersed colloidal dyes, such as fluorescein and naphthol yellow, diffuse into the vascular wall without changing the latter's status of contraction (Zettler). Petroff injected colloidal solutions of trypan blue and lithium carmine into rats and rabbits and found that these colloidal dyes penetrate first into the outer part of the media, then into the inner part and finally into the middle part, indicating that the dyes enter the vascular wall not only through the vasa vasorum but also from the vascular lumen by penetrating the intima. When vessels were rinsed with these vital dyes, only the inner part of the media and the inner elastic membrane became stained. Colloiddally dispersed dyes present in the plasma thus enter the vascular wall in the course

of the normal nutritive processes of these tissues. Dye solutions injected intravenously into rabbits previously given injections of epinephrine hydrochloride accumulate in the injured necrotic and calcified parts of the vascular walls, indicating that the penetration of injured tissues by colloidal material present in the plasma is accentuated. Okuneff confirmed these observations, using trypan blue, which he injected into cats and dogs. He found that the dye caused imbibition of the vascular wall and accumulated in sites where Amschkow noted the localization of lipid spots. The aortas of rabbits, guinea pigs, rats and mice, on the other hand, did not exhibit this focal distribution of the dye but showed diffuse imbibition. Glasunow then rinsed the aortas of rats, guinea pigs and rabbits with a solution of trypan blue and noted that after prolonged rinsing the posterior wall of the rat aorta was more intensely stained than the anterior aspect and that the orifices of the branches stood out because of their more pronounced staining. Boysen showed in experiments on rabbits with a Clark-Sandison window in the ear that India ink particles did not enter the endothelial cells of the capillaries, while trypan blue remained in the blood in a state of colloidal dispersion. Precipitation of the dye occurred only when the dye was taken up by perivascular histiocytes. The endothelial cells remained free from dye particles.

The reported experimental data provide definite proof that colloiddally dispersed matter present in the plasma can enter the normal vascular wall. Since the causal role of colloidal plasmatic disturbances in the production of the atheromatous or the atherosclerotic type of arteriosclerosis can be most readily and convincingly demonstrated by the evidence obtained with exogenous macromolecular carbohydrates, as they are normally not present in the blood and after their introduction do not participate in any physiologic process, the experimental atheromatosis and atherosclerosis elicited by the parenteral introduction of these agents will be presented first.

**EXOGENOUS DISTURBANCES**—Solutions of polyvinyl alcohol, methyl cellulose, pectin and acacia form emulsions with plasma. As these agents display distinct film-forming properties they apparently coat the surface of the erythrocytes and thus interfere with the speed of oxygenation of these cells (Hueper, Martin and Hueper, Christie, Phatak and Olney). Their introduction into the blood elicits the hematic macromolecular syndrome, which includes increased sedimentation and pseudoagglutination of the erythrocytes and colloidoclastic leukopenia, as well as reticuloendothelial and parenchymatous

thesauroses similar to those produced by cholesterol and other lipoids. While polyvinyl alcohol and methyl cellulose are relatively stable and chemically comparatively inert, acacia and particularly pectin are unstable and depolymerize in solution, especially on heating. In solutions of pectin this process apparently proceeds so rapidly and extensively on heating that autoclaved solutions of pectin do not give rise to atheromatous changes, as the pectin molecules have been broken down to molecules of such a small size as to permit their ready passage through biologic membranes. Macromolecular carbohydrates of the mentioned types which elicited atheromatous lesions had an average molecular weight ranging from 32,000 to 200,000. It may be pointed out that these substances form straight chain molecules (polyvinyl alcohol, methyl cellulose, pectin) or branched chain molecules (acacia).

*Polyvinyl Alcohol Atheromatosis*—Experimental studies (Hueper, Hueper, Landsberg and Eskridge, Young, Mulay and Christie, Katzenstein, Winternitz and Meneely, Foster and Jenkins) on dogs, rabbits and rats given intravenous and intraperitoneal injections of aqueous solutions of polyvinyl alcohol have shown that this material is retained for prolonged periods in the blood plasma and is stored in various organs (in the reticuloendothelial cells and histiocytes of the liver, the spleen, the kidneys, the lungs, the pancreas, the heart, the intestine, the bone marrow, the adrenal glands and the lymph nodes, in the endothelial cells of the aorta and the pulmonary, renal, coronary, cerebral, carotid and femoral arteries and glomerular tufts and in the glia and ganglion cells of the brain).

Points of resemblance between cholesterosis and retention of polyvinyl alcohol (which may be termed polyvinylolysis) are supplied by the following facts. Polyvinyl alcohol, just like cholesterol, is chemically a relatively inert substance which forms with the plasma a finely dispersed emulsion, paralleling the so-called protective qualities of cholesterol, based on the high resistance of this substance to enzymatic degradation and on its impermeability to many agents, polyvinyl alcohol displays comparatively marked chemical stability, refractoriness to the enzymes of normal tissues and impermeability to fats, oils, greases and gases. Because of its macromolecular nature and the lack of adequate means of degradation, polyvinyl alcohol is retained in the blood over long periods and tends to coat the inside of the vessels and the surface of the erythrocytes with a film. This film is either the result of a coalescence of fine droplets of polyvinyl alcohol or is the product of a precipitation of this sub-

stance in the interface between the plasma and the intima, as the solubility of polyvinyl alcohol depends on concentration of salts and other physico-chemical factors

The formal genesis of the atheromatosis due to polyvinyl alcohol also follows closely the pattern displayed by that due to cholesterol. The endothelium of the elastic vessels takes up the foreign matter from the blood and is transformed thereby into large, swollen foam cells. Following the reactive proliferation of the damaged endothelial cells which result in the formation of more or less thick cushions of foam cells, and subsequent to the penetration of the media by the polyvinyl alcohol, foam cell endothelial histiocytes invade the inner part of the media. The accumulation of polyvinyl alcohol in the endothelial histiocytes and in the interstices of the media is followed by the appearance of small numbers of fatty granules in the endothelial foam cells and in the muscle cells of the media and in the polyvinyl alcohol infiltrating these parts of the vascular wall. While the fatty substances found in the endothelial lining may be derived from the blood, those present in the media are apparently the result of phanerosis and cellular decay. The affected, usually markedly loosened, parts of the media reveal muscular degeneration and hyalinization as well as disintegration of the elastic fibrils. In animals given moderate amounts of polyvinyl alcohol these changes are restricted to the inner third of the vascular wall. However, in animals with severe polyvinylolysis there occur similar focal foam cell accumulations throughout the entire vascular wall, especially in the perivascular region of the vasa vasorum, which show under such conditions foam cell swelling of their endothelium also. The media of the organic muscular arteries often exhibits in addition to similar endothelial changes marked vacuolation of the muscle cells, the vacuoles being filled with globules of polyvinyl alcohol. It may be mentioned that the atheromatosis due to polyvinyl alcohol not infrequently affects the aortic leaflets by direct extension of the foam cell endothelial proliferation from the basal parts of the aorta upon the valves. Occasionally, a more or less thick coating of foam cells covers the inside of the left auricle and permeates its entire wall. Necrosis, hemorrhages and newly formed capillaries are found in some of the more abundant foam cell proliferations.

The atheromatosis induced with polyvinyl alcohol thus closely resembles in developmental as well as in morphologic respects the corresponding atheromatous change of the larger vessels and the lipoid degeneration of the media of the

arterioles present in human atheromatosis. Its correspondence with the experimental cholesterol atheromatosis is equally close not only as to development and morphology but as to local distribution, since the ascending portion of the aorta of the animal given polyvinyl alcohol exhibits more marked atheromatous lesions than the abdominal part. On the other hand, the atheromatosis due to polyvinyl alcohol does not display the species-specific limitation possessed by experimental cholesterol atheromatosis. This distinction of the polyvinyl alcohol atheromatosis is evidently due to the fact that no species is provided with a metabolic mechanism capable of disintegrating this synthetic, foreign macromolecular material.

In contrast to cholesterol atheromatosis in man the atheromatosis due to polyvinyl alcohol does not show any tendency toward calcification in lesions up to three months old. However, a calcifying type of polyvinyl alcohol atheromatosis can be elicited by combining parenteral administration of polyvinyl alcohol with oral ingestion of excessive amounts of vitamin D. Puppies 2 to 3 months old receiving 25 cc. of a 5 per cent solution of polyvinyl alcohol intravenously twice a week and 50,000 to 250,000 units of vitamin D daily by mouth until a total of 8 to 10 million units has been given during a period of fifty-five days show, in addition to extensive polyvinyl alcohol atheromatous lesions and scattered medial calcifications in the aorta, partially calcified atheromatous foci in the aorta, pancreatic artery and, most extensively, in the left auricle. The large platelike calcium incrustations of the thickened foam cell endocardium affect mainly the necrotic portions and are located in that part of the heart where the blood has a lowered carbon dioxide tension favoring the precipitation of calcium salts (Wells).

While these observations are of distinct importance from the standpoint of pathology, the information which they yield in regard to the causal genesis of atheromatosis and arteriosclerosis is of even greater significance. The data presented permit the conclusion that polyvinyl alcohol present in the blood as a finely dispersed emulsion and coating the vascular endothelial lining of the blood vessels interferes with the normal and adequate exchange of the various gaseous and liquid nutritive substances and waste metabolites between the blood and the inner third of the vascular walls. The resulting cellular injury is intensified when the polyvinyl alcohol is taken up by the endothelial cells and infiltrates the vascular wall, causing further clogging of the normal filtration membrane. The reactive

proliferation of the endothelial cells and their foam cell transformation produce atheromatous lesions. The physicochemical qualities of polyvinyl alcohol films toward fatty substances and the absence of any appreciable amount of fat-stainable material in these lesions, as well as of any hypercholesteremia, exclude any significant role of lipids in the development of these vascular changes.

*Methyl Cellulose Atheromatosis*—Methyl cellulose, another highly polymerized carbohydrate, elicits when injected into dogs and rabbits hematic and organic reactions which closely resemble those produced by polyvinyl alcohol (Hueper, Hueper and Ichniowski, Katzenstein, Winternitz and Meneely). Like polyvinyl alcohol, it is a hydrophilic colloid with film-forming qualities which produces when introduced intraperitoneally or intravenously into the blood the hematic macromolecular syndrome and is retained there over long periods, forming an emulsion with the plasma. It is deposited in the reticulo-endothelial cells and histiocytes of the lung, the heart, the liver, the spleen, the kidneys, the adrenal glands, the lymph nodes, the choroid plexus, the hypophysis and the small intestine, in the parenchymatous cells of the liver and in the endothelial cells of the elastic and muscular arteries and of the glomerular tufts.

The early vascular change usually consists of a focal foam cell transformation of the endothelial lining. This is sometimes preceded by a localized budlike or cushion-like proliferation of the endothelial cells. In some instances, just as in polyvinyl alcohol atheromatosis, the endothelial lining appears to be intact and an accumulation of foam cells is found beneath it, resting on the internal elastic membrane. In somewhat older intimal lesions there appears a proliferation of fibroblasts and of hyaline matter together with a decrease in the number of foam cells. With severe degrees of methylcellulosis, foam cells and free methyl cellulose-plasma emulsion penetrate the internal elastic membrane and invade the media. A foam cell transformation of the endothelium of the large vessels and foam cell accumulations around the vasa vasorum are then seen. Ultimately the entire vascular wall may be converted into a thick mass of foam cells under complete destruction of the media. Such changes occur particularly frequently in the myocardial and uterine arteries. Characteristic of the atheromatosis due to methyl cellulose is the appearance of focal and sometimes extensive hyalinizations and calcifications in the media beneath the foam cell intimal cushions. An isolated area of diffuse severe calcinosis of the media is occasionally seen in

medium-sized muscular arteries without any intimal foam cell reactions being present. This type of arterial reaction resembles closely the Monckeberg type of medial calcinosis in man. Its occurrence under these circumstances indicates that colloidal plasmatic disturbances may play an important role in the production of such lesions.

The character of the vascular reactions elicited was identical regardless of the average molecular weight of the methyl cellulose used. Seven types of methyl cellulose were employed in the experiments, ranging in molecular weight from 32,000 to 140,000. These observations furnish another illustration suggesting that substances circulating in the blood and similar in physicochemical properties (chemical inertness, colloidal dispersion, nonpenetrability to nutritive and metabolic elements) are capable of producing atheromatous arterial lesions by impairing the oxygenation and nutrition of the vascular wall when deposited or precipitated on and in the vascular walls.

*Pectin Atheromatosis*—Additional confirmation of this concept was obtained when solutions of pectin were injected into dogs and rabbits (Hueper). Pure citrus pectin, which has an average molecular weight of 200,000 and which consists of polymerized galacturonic acid molecules with various additional groups, elicits the hematic macromolecular syndrome and thesaurotic phenomena in the liver cells, the reticulo-endothelial cells and histiocytes of the lungs, the liver, the spleen, the kidneys, the hypophysis, the choroid plexus and the bone marrow and in the endothelial cells and walls of the large and small arteries and of the glomerular tufts.

The primary vascular changes consist of small focal proliferations of the endothelial cells which stud the vascular lining like small seeds. These primary reactions grow to small elevated endothelial cushions which show a scanty accumulation of foamy cytoplasmic matter. Later larger cushions of foam cells appear which finally invade the media. This invasive response is associated with the appearance of large mononuclear cells within the cushions and in the media. There is a definite tendency of the larger cushions either to break down and ulcerate or to degenerate with disintegration of the foam cells, proliferation of fibroblasts and deposition of calcium salts. The final stage of this development is represented by hyaline and locally calcified thickenings of the intima. Hyaline degenerations of the media are found in the aorta, the pulmonary artery and the renal arteries.

These observations, which confirm those made in connection with polyvinyl alcohol atheromatosis and methyl cellulose atheromatosis, suggest,

moreover, that the speed with which degenerative, hyalinizing and calcifying changes occur in atheromatous lesions depends on the metabolic stability of the causal atheromatogenic substance

*Acacia Atheromatosis*—Acacia, another chemically complex, highly polymerized hydrophilic macromolecular carbohydrate, elicited when injected intravenously into dogs and rabbits a hematic macromolecular syndrome and storage reactions in the liver cells, the reticuloendothelial cells and histiocytes of the spleen, the lymph nodes, the kidneys, the adrenal glands and the lungs and in the endothelial cells of aorta and the glomerular tufts (Hueper). The foam cell intimal thickenings, however, were infrequent and small, while medial hyalinizations and calcifications were somewhat more frequent. The relatively moderate degree of the atheromatous responses observed in dogs and rabbits in spite of prolonged treatment with large amounts of acacia solution in high concentration is probably attributable to the instability of the macromolecules of acacia and the resulting fleeting nature of the disturbances in the colloidal plasmatic equilibrium. These observations are of clinical importance, as large amounts of acacia solution are injected intravenously into patients with nephrosis to counteract the existing hypoproteinemia, which is usually associated with hyperlipemia and hypercholesteremia, giving rise to atherosclerotic reactions.

*Glycemic Vascular Reactions*—In glycogen storage disease (von Gierke's disease), which is characterized by the hematic macromolecular syndrome and the deposition of glycogen in the liver, the heart, the kidneys and the brain, hyperglycogenemia is accompanied by hypercholesteremia (van Creveld, Wagner, von Gierke, Krakower, Beumer and Loeschke, Unshelm, Siegmund). Degenerative, sclerosing or atheromatous vascular lesions have not been observed in the relatively few cases of this disease, affecting mainly young children, which have come to autopsy. There are, however, several cases of this disease on record in which glycogen was found in unusual amounts in the endothelium and muscularis of small arteries (von Gierke, Kimmelstiel, Humphreys and Kato, Antopol, Heilbrunn and Tuchman, Hertz and Jecklen, Wolff). Hueper injected glycogen solution intravenously into 2 dogs daily for a period of thirty days and observed reactions of the blood conforming with the hematic macromolecular syndrome, focal edematous intimal swellings of the aorta and localized hyalinizations of the media of the aorta and some coronary and renal arteries. In view of the small number of animals used, the short duration of

the experiment and the relatively small amounts of glycogen injected, the glycogenic origin of these moderate vascular reactions is merely suggestive.

*Summary*—The observations recorded show that repeated intravenous or intraperitoneal injections of excessive doses of various highly polymerized macromolecular carbohydrates which are used or proposed for use as plasma substitutes elicit, in addition to colloidal plasmatic disturbances, atheromatous arterial reactions which are morphologically identical with those seen in man and produced in rabbits by the feeding of cholesterol. The similarity between these experimental carbohydrate atheromatoses and the lipoidal ones extends to the topographic distribution of the atheromatous reactions within the vascular tree, the physicochemical conditions of the plasma underlying them and their relation to the causal mechanism responsible for the development of mediocalcinosis. The hyaline-calcinotic intimal thickenings representing the cicatricial end results of the atheromatous changes, on the other hand, are indistinguishable morphologically from similar lesions originating on a different causal basis.

#### COLLOIDAL PLASMATIC DISTURBANCES A CARBOHYDRATIC TYPE

- Antopol, W., Heilbrunn, J., and Tuchman, L. R. *Am J M Sc* **188** 354, 1934  
 Beumer, H. *Klin Wchnschr* **16** 654, 1937, *Verhandl d deutsch path Gesellsch* **31** 188, 1938  
 Boysen, B. *Frankfurt Ztschr f Path* **45** 487, 1933  
 Christie, A., Phatak, N. M., and Olney, M. B. *Proc Soc Exper Biol & Med* **32** 670, 1935  
 van Creveld, S. *Klin Wchnschr* **12** 529, 1933, *Medicine* **18** 1, 1939  
 Foster, R. H. K., and Jenkins, L. *Arch Path* **37** 279, 1944  
 von Gierke, E. *Beitr z path Anat u z allg Path* **99** 369, 1937  
 Glasunow, M. *Virchows Arch f path Anat* **261** 837, 1926  
 Hueper, W. C. *Arch Path* **28** 510, 1939, **31** 11, 1941, **33** 1 and 267, 1942, **34** 34, 1942, **36** 381, 1943, *Am J Path* **18** 895, 1942, **20** 211, 1944  
 —and Ichniowski, C. T. *J Pharmacol & Exper Therap* **78** 282, 1943  
 —Landsberg, J. W., and Eskridge, L. C. *ibid* **70** 201, 1940  
 Katzenstein, R., Winternitz, M., and Meneely, J. *Yale J Biol & Med* **16** 561, 1944  
 Kimmelstiel, P. *Beitr z path Anat u z allg Path* **91** 1, 1933  
 Krakower, C. *J Pediat* **9** 728, 1936  
 Martin, G. J., and Hueper, W. C. *Proc Soc Exper Biol & Med* **49** 452, 1942  
 Okuneff, N. *Virchows Arch f path Anat* **259** 685, 1926  
 Petroff, J. R. *Beitr z path Anat u z allg Path* **71** 115, 1922  
 Siegmund, H. *Verhandl d deutsch path Gesellsch* **31** 150, 1938



- Wagner, R. *Ergebn d inn Med u Kinderh* **53** 586, 1937
- Wolff, K. *Beitr z path Anat u z allg Path* **97** 289, 1936
- Young, W. R., Mulay, A. S., and Christie, A. J. *Lab & Clin Med* **27** 1131, 1942
- Zettler, L. *Arch f exper Path u Pharmakol* **185** 141, 1937

#### COLLOIDAL PLASMATIC DISTURBANCES B LIPOIDAL TYPE

Intimal lipoidal deposits, which in their pure form are represented by atherosclerosis or atheromatosis and which appear in connection with fibrosing and hyalinizing processes as atherosclerosis, are the most frequent lesions of the large elastic arteries, but occur also in the larger muscular arteries (coronary, cerebral, pulmonary and renal arteries and, less often, splenic and mesenteric arteries and branches of the celiac axis) (Ophuls, Bork, Sappington and Horneff, Wolkoff, Veise). Arteries of the extremities contain, as a rule, only minor or moderate atheromatous lesions (Sappington and Horneff, Hesse, Kusnetzowski) unless special causal factors, such as diabetic hypercholesteremia, prevail (Hines and Barker, Sappington and Fisher). However, even under these conditions mediocalcinosis remains the characteristic lesion of the arteries of the lower extremities. The significance of atheromatous changes in the aorta and the coronary arteries is illustrated by a statement of Leary to the effect that practically all of the arteriosclerotic lesions of the aorta and the coronary arteries are of the fatty form. Haythorn, Taylor, Ciago and Burrier mentioned, however, that during the advanced stage of aortic atherosclerosis there occurs overlapping of type lesions (atheromatous cysts, diffuse mediocalcinosis and plaque formation).

**THEORIES AS TO THE ORIGIN AND ETIOLOGIC IMPORTANCE OF THE LIPOIDS IN THE ATHEROMA OF THE ARTERIAL INTIMA**—The role of cholesterol in the genesis of the intimal atheromatosis and of the intimal and medial calcinotic reactions often associated with the lipoidal reactions of the arteries and the origin of this substance have remained highly controversial subjects. Some (Virchow, Thomas, Faber, Lange, Staemmler, Moschowitz, Beitzke) have asserted that cholesterol together with other fats is liberated in fibrous-hyaline intimal thickenings during the course of necrobiotic processes and thus represents merely a by-product of the regressive phase of intimal arteriosclerotic lesions. Beneke apparently held a similar opinion when he contended that intimal lipoidosis is the result of continued vascular tension or pressure. The presence of cholesterol deposits in the vascular

lesions, according to the necrobiotic theory, is purely incidental. Many others (Aschoff, Ribbert, Marchand, Burger and Schlomka, Lubasch and others) have held that as a part of the colloidal aging process a loosening of the connective tissue ground substance of the intima precedes the appearance of cholesterol, which enters the altered intima together with the plasma from the vascular lumen and is retained there especially by the mucoid matter, which possesses a particular affinity for cholesterol (Schultz, Aschoff, Hueck, Saltykow, Stumpf, Torhorst, Voigt, Ssolowjew, Nordmeyer). This process of intimal imbibition of plasma with deposition of cholesterol is facilitated by hypercholesteremia. Hirsch and Weinhouse, and Landé and Sperry, again emphasizing the importance of primary changes in the intimal tissues for the precipitation of cholesterol, recently stated that cholesterol has no specific significance in the production of atheromatosis but acts indirectly through its influence on the physicochemical state of the plasmatic lipoids as a whole. The role of cholesterol in the production of atheromatous changes, according to Aschoff's theory of colloidal aging, is mainly a passive one, conditioned by primary colloidal senescing changes in the vascular wall.

Leary has recently modified this theory by claiming that cholesterol is introduced into the subendothelial tissue by cholesterol ester-containing lipophages that penetrate the intact endothelial lining. These cholesterol-carrying lipophages which are specifically attracted by the arterial intima are derived from desquamated foam-cellular reticuloendothelial cells of the liver, the adrenal glands and the lungs and show, according to Leary, all conceivable transitions between lymphocytes, large mononuclear cells and large lipophagic cells when circulating in the blood. Endothelial cells and intimal fibroblastic cells are primarily free from cholesterol. The alleged affinity of the vascular wall for these cholesterol ester-phagic cells is highly selective, as phagocytes containing other substances are not attracted by the arterial walls. Leary claimed further that in early youth the cholesterol esters are transferred from the lipophages to the proliferating fibroblasts in the intima, which undertake their metabolic destruction without the formation of collagen. In later life this quality of the fibroblasts is increasingly lost by the arteries except in the ascending part of the aorta. As there occurs then no proliferation of fibroblasts, the foam cells accumulate in the intima and give rise through necrobiotic disintegration to the formation of atheromas. Cholesterol assumes in this lipophagic theory a place secondary to the

specific "affinity of the vascular tissues, as it is the object of attraction," while the tissue reactivity is the important and variable factor, accounting for the different anatomic types of lesions characterizing various age periods.

A fourth theory was advanced several years ago by Winternitz, who asserted that the primary event in the evolution of an atheroma is represented by the penetration of newly formed capillaries extending either from the vascular lumen or from the vasa vasorum into the intima. Following the occurrence of hemorrhages from such intimal capillaries, cholesterol contained in the plasma and liberated from the disintegrating blood cells appears in the decomposing hemorrhagic foci and thus gives rise to the ultimate formation of necrotic intimal thickenings containing cholesterol and fat. The role of cholesterol in the formation of atheromas is evidently only a fortuitous one, according to this capillarization theory.

Anitschkow and his many followers (Beneke, Rosenthal, Ciamei), on the other hand, maintained that the deposition of cholesterol in the arterial intima is the result of hypercholesterolemia. The plasmatic cholesterosis leads in this theory to vascular changes (Koppenhofer) as the cholesterol is taken up by the endothelial cells and subendothelial phagocytic cells, which are transformed thereby into foam cells, while at the same time the lipid-containing plasma seeps into the interstices of the intima giving rise to accumulations of extracellular lipoidal material, especially along the internal elastic membrane, which acts as a filtration barrier for the plasmogenic tissue juices. Quantitative fluctuations in the cholesterol content of the plasma, according to this hypercholesteremic theory, appear as the significant factor in the genesis of atheromatosis.

Hueper recently came forth with a new theory in which it was contended that the atheromatous process is initiated by an imbalance of the plasmatic colloid equilibrium causing the precipitation of a cholesterol film on the intima. Such a film, it was claimed, interferes with the proper exchange of gases and nutritive substances between the blood and the vascular wall, as cholesterol in a precipitated form is chemically relatively inert and impermeable to many substances. The resultant injury to the endothelial cells causes increased permeability of the endothelial lining and proliferation of the endothelial cells, which take up cholesterol and are thereby transformed into foam cells. A similar conversion into foam cells takes place in the intima where cholesterol contained in the plasmatic fluid penetrating into the subendothelial space is phagocytosed by histiocytes. The in-

stability of the plasmatic colloidal solution of cholesterol which starts this chain of events in the vascular wall may be due to quantitative imbalance in the cholesterol content (hypercholesterolemia), or it may result from an increase of substances in the plasma which decrease the dispersion of cholesterol and therefore favor its precipitation or from a decrease of substances which enhance the dispersion of cholesterol. The physicochemical properties of cholesterol decidedly influencing the oxygenation and nutrition of the vascular wall are significant factors in the production of cholesterol atheromatosis, according to Hueper's anoxemic theory, which is based on the analogous conditions present in the atheromatosis elicited by polyvinyl alcohol, methyl cellulose, pectin and acacia.

Inasmuch as the relative merits of the various theories cannot be properly evaluated without giving due consideration to normal and abnormal cholesterol metabolism, a brief resumé of the pertinent information on this subject is given first.

Cholesterol present in the blood and in the tissues is in part synthesized in the body, in part it originates from the animal food ingested, such especially as eggs, butter, milk, laid, brain and meat. Phytosterols contained in vegetable matter are not absorbed by the animal organism (Schonheimer and Yuasa, Schonheimer, von Behring and Humel, Schonheimer). Blood stated that milk contains about 0.2 Gm of cholesterol per liter, egg yolk about 1.9 Gm, and skeletal muscle 0.07 to 0.09 Gm per kilogram. Hueck estimated that a baby when breast fed consumes daily about 49 to 97 mg of cholesterol, and when on a mixed diet, about 62 mg. He estimated that adults on a fat-poor diet receive between 39 and 109 mg daily, those on a mixed diet between 200 and 362 mg and those on a high fat diet up to 1,400 mg. Milk contains cholesterol mainly in the form of esters, whereas meat and brain contain mostly free cholesterol. Cholesterol added in a dry form to food poor in fat is not absorbed by the intestinal mucosa. Foodstuffs rich in fat or oil, on the other hand, dissolve cholesterol, which is emulsified in the intestine through the action of bile acids, particularly by desoxycholic acid and its alkaline salts, less by taurocholic and glycocholic acid (Wieland and Sorge). In the absence of fatty acids, free and esterified cholesterol does not pass the intestinal mucosa. The lymph of the thoracic duct contains 25 to 30 per cent of the total cholesterol in the free form, the balance is in the ester form, bound to fatty acids (Blood, Hueck) mainly of the unsaturated reactive type (Blood, Blake and Bullen). A similar ratio (2:1) of esterified cholesterol to free cholesterol is noted in the plasma, which normally contains from 130 to



230 mg of cholesterol in 100 cc, while the bile contains from 39 to 61 per cent free cholesterol. Cholesterol is not readily degraded metabolically (Burger and Schlomka) and is excreted with bile, sebum, milk, intestinal secretion and urine and through the skin, the lungs and the bronchi.

Both free and esterified cholesterol are anisotropic and occur in the plasma in colloidal dispersion (Bruger). The plasmatic cholesterol represents the bulk of the total blood cholesterol content as only relatively minor amounts of cholesterol are contained in leukocytes and erythrocytes. Since cholesterol is a hydrophobic colloid, it is held in colloidal dispersion by the presence of hydrophilic colloids (lecithin, protein) (Degwitz, Hirsch and Weinhouse). Remesow noted that the labile hydrophobic cholesterol mycelle can be converted into a hydrophilic one by the addition of protein, carbohydrate or fat. Cholesterol-plasma protein addition products occur in the plasma (Bennhold). The close physicochemical relations between these two substances are demonstrated, moreover, by the fact that 70 per cent of the total cholesterol is precipitated with the protein by the salting out method. Weinhouse pointed out that cholesterol is bound to albumin by secondary valences or is combined with it through opposite electrical charges holding protein micelles and high molecular aggregates of lipid molecules together or becomes associated with protein by simple physical enmeshing. This attraction between cholesterol and protein aggregates results in a uniform dispersion of discrete lipid molecules throughout the protein micelles. It is apparently for these reasons that Bruger found in ultrafiltration experiments on transudates and exudates that membranes which were not permeable to proteins did not permit the passage of cholesterol, indicating thereby that cholesterol exists in these plasma-like fluids in large molecular aggregates approximating the protein micelles in size and resembling them in other physical properties. The firmness of the binding of cholesterol to protein can be varied *in vitro* by the addition of small amounts of dextrose or salts to serum and appears to be changed after the intravenous injection of crystalloid solutions, according to Handovsky. While molecular cholesterol is chemically relatively nonreactive, colloiddally dispersed cholesterol is chemically highly active, displaying strong reducing qualities and oxidase activity (Remesow).

Lipschutz, on the other hand, suggested that cholesterol may impair oxidative processes. Cholesterol, which in contrast to proteins and phosphatides has practically no electric charge, acts electrically as an insulating agent which inhibits the permeability of membranes and thus

the movement of ions to a degree corresponding with the amount of cholesterol present in the interface. Wacker and Hueck pointed out that cholesterol esters accumulate at sites of high carbon dioxide tension and proposed that a relation exists between this process and the inhibition of oxidation. Observations made by Martin and Hueper on the reduced speed of oxygenation of the erythrocytes of hypercholesteremic rabbit support this concept. The biologic activity of cholesterol thus depends in part on its effect on membranes (Thannhauser, Hueck, Epstein).

It may be mentioned in this connection that hypercholesteremic thesaurosis of the spleen results in the development of an anemia (Okey and Greaves, Member, Ehrlich and Bruger) which has apparently the same hyperplastic splenic genesis as that seen in other lipoidal and carbohydrate thesauroses of this organ (Hueper). The anemogenic effect of cholesterosis, however, must be distinguished from the transitory reduction in erythrocytes associated with alimentary hyperlipemia and caused by the increased erythrocytic fragility and destruction elicited by the action of fatty acids and soaps (Johnson, Freeman, Longini and Loewy, Longini and Johnson, Dupee, Johnson, Marchello, Wilner and Freeman). The hyperlipemia often accompanying hypercholesteremia may accentuate, on the other hand, the development and the severity of the splenogenic anemia by increasing the rate of destruction of erythrocytes.

The stability of the colloidal cholesterol solution is highly important. It is influenced by the cholesterol-lecithin ratio (Medvei, Tannenberg, Hueck), the hydrogen ion concentration, the amount of albumin, the bile acids and other factors. While SS and SH groups in the protein molecules decrease the precipitability and increase the dispersion of cholesterol,  $\text{NH}_2$  groups have an adverse effect on these conditions (Durr). Kimmelstiel noted that the addition of galactosides to mixtures of cholesterol and cephalin or lecithin favors the precipitation of cholesterol. Addition of dextrose exerts a similar effect on cholesterol sols. An increase of cholesterol or other hydrophobic colloids therefore causes instability of the colloidal dispersion of cholesterol and its agglomeration into larger particles. This reaction is responsible for the appearance of opalescence in hypercholesteremic plasma. Free cholesterol reacts, moreover, with various toxins and hemolytic substances (saponins, digitonin), forming thereby addition products with lowered stability (Gugaut, Ransom, Ehrenthel and Weis-Osborn, Kollert, Lee and Tasi, Kyes and Sachs). Through its esterification with unsaturated fatty acids it may par-

ticipate in the metabolism and transport of these substances (Bloor, Hurxthal and Hunt)

Burger found that the arterial blood contains regularly about 11 per cent more cholesterol than the venous blood and concluded therefrom that the blood lipoids undergo during their passage from the arterial to the venous side quantitative and qualitative changes. This observation agrees in principle with those made by several investigators concerning the arrest of cholesterol in the pulmonary capillaries, where cholesterol is temporarily kept and esterified (Remesow and Tavaststerna, Abelous and Soula, Zinseiling, Engel-Cserna, Kaufer). Remesow and Tavaststerna, however, pointed out that apparently only the rabbit's lung metabolizes the arrested cholesterol injected intravenously, while the dog's lung treats it like a foreign body. This species-specific difference in the reactivity of the lungs of herbivorous and carnivorous animals may account for the fact that Shillito, Bidwell and Turner did not find any significant variation in the cholesterol content of the blood before and after passage through the lungs. Kovats noted that the blood coming from the spleen contained a higher fraction of esterified cholesterol than that in the blood going to this organ.

The validity of the necrobiosis theory of Virchow and others rests mainly on the observation that in marked fibrous and hyaline primary thickenings of the intima the development of necrotic processes is associated with the appearance of fat, including cholesterol, and of usually only a small number of foam cells. This fact, however, does not justify the conclusion that the majority of fat-containing intimal lesions are of this genesis. There are a large number of reliable observations attesting that the deposition of fat, usually together with some fibroblastic proliferation, is a primary reaction of the arterial intima. Chemical analyses of the fat content of atheromas have shown, moreover, that during the early stages of atheromatosis the lipid and lipid composition of the atheromatous content (55 to 60 per cent esters, 23 to 26 per cent free cholesterol, 20 per cent other lipids) is similar to that of the blood. Among the various analyses made (Wells, Schonheimer, Kimmelstiel, Rosenthal, Page and Menschick, Lehnheit, Meeker and Jobling, Zeek, Lande and Sperry, Hirsch and Weinhouse, McArthur) those of Schonheimer, Hirsch and Weinhouse, Lande and Sperry, and McArthur revealed that the ratio of free cholesterol to esterified cholesterol in the atheromatous matter is about the same as that of the plasma, indicating the hematogenous origin of the lipoidal material. McArthur noted, moreover, that the fatty acids present in the esters are oleic, palmitic, linoleic and arachidonic acid and some

other petroleum-insoluble unsaturated acids. The most striking difference between the fats in atheromas and those in normal tissue is in the neutral fats fraction, according to McArthur, as the fats of atheromas are 13 to 27 per cent neutral fats, whereas the proportion of these in the fats of normal plasma and blood cells is 35 to 41 per cent, suggesting that glycerides either are not absorbed from the plasma or undergo subsequent decomposition in the intima. The results reported by Hirsch and Weinhouse support the last-mentioned mechanism, as they found that the cholesterol content increases and the neutral fat and fatty acid contents drop sharply during a more advanced stage of the atheromatous development and undergo during the final stage a reversal of these quantitative relations.

The original colloidal aging theory of Aschoff does not take proper account of the fact that the chemical, physical and morphologic changes occurring in arteries with age are independent of atherosclerosis. The presence of lipid spots in the aortic intima of babies and juvenile persons, moreover, demonstrates clearly that the changes of aging are not essential for the deposition of lipoids in the intima, while the rapidly increasing number of observations on the frequency of typical atherosclerosis, particularly of the coronary arteries, in young adults suffering from diseases accompanied by disturbances of the cholesterol content of the blood provide additional evidence against the validity of this theory. The experimental production of the so-called arcus senilis of the cornea resulting from the precipitation of cholesterol in this tissue and attributed by Aschoff to senile dehydration of the colloids has been accomplished in cholesterolized rabbits by Schonheimer, Versé, Kolen and Rosenthal. Joel, moreover, demonstrated the occurrence of arcus senilis in young and middle-aged adults having an increase in blood cholesterol but showing no evidence of premature senescence in any other organ. As the experimental arcus senilis is partly reversible, it is likely that any local tissue changes in the cornea are likely to be secondary and not primary and that the precipitation of cholesterol in this nonvascularized tissue, resembling in this respect the inner part of arteries, is based on physiologic conditions and not on pathologic ones. The arguments of Landé and Sperry and of Hirsch and Weinhouse in favor of unknown factors in the tissues of the blood vessels which allegedly favor the deposition of lipoids are based on the observation that the atherosclerotic lesions develop during adult life without any appreciable elevation of blood cholesterol or obvious abnormality of the blood lipids. It will be shown later in the discus-

sion of the various hypercholesteremic conditions that these contentions are not generally applicable since the evidence available on this matter does not support such generalizations. Hirsch and Weinhouse, as well as Kimmelstiel, concede, moreover, that the main cause of precipitation of the lipid, in cholesterolized rabbits as well as in man with essential xanthomatosis may be of hypercholesteremic-hyperlipemic (cholesteatotic) origin and not related to any tissue factor of senile or undetermined nature.

The lipophagic theory of Leary is based on several assumptions and unconfirmed observations. One of the fundamental contentions of his theory deals with the presence of foam cell lipophages in the blood which are specifically attracted to the arterial wall. While it is possible that a few such cells circulate in the blood after being released from their normal sites in the spleen, the liver, the adrenal glands, and the lungs, there is no additional confirmatory evidence from any other source that these cells are of any significance in the production of atheromas, i.e., that they invade the subendothelial tissue by penetrating through an intact endothelial lining. This is an important fact weighing against Leary's theory in view of the huge amount of experimental work that has been done on the production of atheromatosis in rabbits and suggesting an incorrect interpretation of anatomic evidence. It is, moreover, not correct that the endothelial lining in experimental atheromatosis is intact, as it can readily be shown not only that foam cell accumulations are present beneath an apparently normal endothelium but that endothelial cells may become transformed into foam cells while the subendothelial tissue remains apparently intact (Hueper). Additional doubts as to the validity of this theory are derived from the fact that the foam cell reticuloendothelial proliferations accompanying several lipidoses (Niemann-Pick disease, Gaucher's disease) do not give rise to atheromatous formations in spite of the fact that at least in Niemann-Pick disease foam cells containing anisotropic material are found in the circulating blood, which exhibits in some instances an elevated cholesterol level (Letterer, Zehnder, Hamperl, Baumann, Smetana, Tropp, Siegmund). The experiments of Hueper with polyvinyl alcohol, methyl cellulose pectin and acacia have definitely shown that the alleged affinity of the arterial wall for foam cells is apparently not restricted to those containing cholesterol esters—assuming for the sake of argument that the concept of Leary concerning the hematic origin of the subendothelial foam cells is correct. The cited experiments of Hueper, as well as subsequent ones performed by

him on rabbits fed cholesterol, have demonstrated, on the other hand, that the endothelial cells, as well as the subendothelial histiocytes, take up the atheromatogenic matter and become converted thereby into foam cells. The relatively low incidence of atheromatosis in the arteries of the lung, where, according to Leary, the foam cells are arrested and the cholesterol is metabolized, speaks against his theory.

The doctrine of Leary concerning the age-conditioned differences in the reactivity of the arterial wall to the cholesterol-containing lipophages is untenable on considering the fact that the same morphologic and topographic types of atheromatosis are observed in the young as in the old in connection with diabetes mellitus, chronic renal disease, essential xanthomatosis and other hypercholesteremic conditions. Whatever differences may exist in this respect between various age groups are attributable to other factors, such as static conditions, endocrine function and the duration of exposure to the causal factors.

The teachings of Winternitz in his vascularization theory are based on the observation that the arterial wall is often quite vascular in and adjacent to the region of atheromatous plaques. It is in general recognized, however, that these capillary proliferations which extend from the vascular lumen and the vasa vasorum into the plaques are of secondary nature, appear only in advanced lesions and are not the cause of the plaque formation (Aschoff, Leary, Schlossman and Geibel, Horn and Finkelstein, Sumikawa, Goldmann, Paterson, Hueper). They are thought to be of reactive nature, intended perhaps for the better resorption and removal of the deposited lipidal or synthetic carbohydrate matter. In commenting on the theory of Winternitz, Scupham, de Takats, Van Dellen and Beck pointed out the following two outstanding weaknesses of this theory.

The first difficulty is that, while the demonstration of anastomosing channels in the intima of normal arteries of different animal species can be accepted, it is a fact that the arteriosclerosis which occurs in such animals is most commonly medial, characterized by degeneration and calcification of this coat and not by atheromatosis, which is an important part of the lesion of simple intimal arteriosclerosis in man. The second difficulty is that these authors have failed to demonstrate anastomosing channels in the intima of the normal human aorta and arteries, yet it is in this very location that simple arteriosclerosis develops in man.

Hughes and Peiry made special mention of the fact that in a case of marked intimal thickening of the aorta in a guil 7 weeks old vascularization was absent, while Horn and Finkelstein noted

that atherosclerosis of the pulmonary artery is relatively rare although the wall of the pulmonary artery is exceedingly vascularized. Peary who studied the distribution of the vasa vasorum in the aorta, stated that atherosclerosis develops in the intima, i. e., in a region farthest removed from the vasa vasorum, although the richer the anastomosing network of the vasa in a given site, the greater is the likelihood that lesions will arise there. It may be pointed out in this connection that the vasa vasorum in the large veins penetrate to the intima (Schieffedecker) but that atherosclerosis of these vessels is quite uncommon and seen only in the presence of severe and prolonged hypercholesteremia and after administration of large amounts of polyvinyl alcohol or methyl cellulose without any evidence of primary intimal vascularization complicated by intramural hemorrhage. Hemorrhages into atherosclerotic plaques have been observed repeatedly in man (Winternitz, Thomas and LeCompte, Waitman, Nelson, Paterson, Horn and Finkelstein, English and Finkelstein) together with the origin of intimal capillaries from the lumens of sclerotic arteries. The observations of Zinserling on organizing cardiac thrombi on the other hand, showed that lipid masses and foam cells appeared in the superficial parts of thrombi which were exposed to the surrounding blood and derived apparently from the infiltrating lipid-containing plasma. The available evidence lends little support to the theory of Winternitz and mainly opposes it.

The hypercholesteremic theory of Anitschkow holds that cholesterol in the form of an emulsion with the lymph infiltrates the vascular wall and becomes adsorbed there to the ground substance and the surface of the elastic fibrils. For the development of the resulting atheromatous lesions it is not essential, according to the available clinical and experimental evidence, that the hypercholesteremia is persistent or that an appreciable increase in the blood cholesterol exists at all as long as there are factors active which cause precipitation of the cholesterol in the intima. Such factors of local type may be of physicochemical nature causing instability of the cholesterol sol, or they may be represented by a relatively slowed metabolism delaying the elimination of this substance, or they may appear in the form of an increased amount of ground substance accentuating the adsorption and retention of cholesterol. The cellular and fibrous proliferative processes are regarded as secondary reactions to the presence of cholesterol (Anitschkow).

These concepts have not remained unchallenged. Thus Hirsch and Weinhouse noted that the specific significance of cholesterol in

experimental atherosclerosis is uncertain, while the role of the increase of the other lipoids and their relation to cholesterol are equally obscure. Hypercholesteremia as the cause of atherosclerosis has not been demonstrated convincingly in man in the opinion of these investigators. Page as well as Kimmelstiel, is equally skeptical in this respect and considers the importance of cholesterol in the genesis of atherosclerosis as much overemphasized and definitely uncertain. In view of these objections against the theory of Anitschkow it appears pertinent to review the relationship which exists between hypercholesteremia and atherosclerosis in man and experimental animals.

**ENDOGENOUS DISTURBANCES ASSOCIATED WITH HYPERCHOLESTEREMIA AND INSTABILITY OF PLASMATIC COLLOIDAL LIPOIDS —*Biliary Obstruction*** — Hypercholesteremia characterized by a relatively low cholesterol ester fraction (30 to 50 per cent of the total cholesterol) is associated with diseases causing obstruction of the bile ducts and thereby interfering with the elimination of cholesterol with the bile (Gardner, Hurxthal and Hunt, Stepp, Burger and Habs, Epstein and Greenspan). The coexistence of chronic jaundice and xanthomatosis has been reported (Pye-Smith, Smith, Schulte, Murchison, Fitcher, Moxon, Gull-Addison, Hertzler). Fagge recorded a case of chronic jaundice and multiple xanthoma of the skin and of the arteries (aorta and pulmonary artery), whereas Gabbe mentioned the occurrence of chronic hepatic disease, tuberous xanthomatosis, angina pectoris and coronary arteriosclerosis.

**Essential Xanthomatosis** — Hypercholesteremia with predominance of the ester fraction accompanies familial essential tuberous xanthomatosis (Thannhauser, Burger, Svendsen, Nékam and Ottenstein, Polano, Letterer, Muller, Guenfeld and Seelig, Bloom, Kaufman and Stevens, Dauksys, Engelberg and Newman, Lane and Goodman). The coexistence of this type of xanthomatosis with cardiovascular diseases affecting often young persons and causing sudden death has been observed (Gwynne, Hess, Svendsen, Lane and Goodman, Lapowski, Lehzen and Knauss, Wise, Wolff, Muller, Engelberg and Newman, Bloom, Kaufman and Stevens, Fagge, Poensgen, Weidman and Boston, Chalator, Weber, Arning, Montgomery, Thannhauser and Magendantz). In some of these cases the diagnosis of xanthoma endocardii was made. In others there were attacks of angina pectoris or there was hypertension related to atherosclerosis of the renal arteries (Engelberg and Newman). In some of these cases as well as in additional ones, the post-

mortem examination showed extensive and not infrequently nodular atheromatous-xanthomatous intimal lesions in the large elastic and muscular arteries in the endocardium (Arning and Lippmann, Harbitz, Lubaisch, Bross, Schulte, Balzer, Hoffmeyer) It is characteristic of the vascular manifestations accompanying this lipoidosis that the atheromas are composed of masses of foam cells (vascular xanthomas) and that particularly the ascending part of the aorta and the coronary and pulmonary arteries are involved The atheromatous changes noted in the pulmonary vessels resemble closely those seen in connection with pulmonary fibrosis caused by chronic empyema and caseous pneumonia, i e, in conditions in which large amounts of lipoidal cytolytic material are liberated (Pagel, Fischer) The foam cell proliferations in the smaller arteries are sometimes so extensive that they occlude the vascular lumens, while they may narrow considerably the lumens of large arteries (Lehzen and Knauss) In such cases foam cells may penetrate through defects of the internal elastic membrane into the media The vasa vasorum of such vessels are often involved by the same process Degenerative changes in even large foam cell proliferations are rare In old lesions there takes place a replacement of the foam cells by fibrous tissue such as is seen in ordinary atheromas

Hypercholesteremia without a shift in the ratio of free cholesterol to cholesterol esters is found in many cases of Hand-Schüller-Christian xanthogranulomatosis (Eppinger, Burger, Strong, Natali, Dauksys, Gross and Jacob) In several cases there was observed intimal lipoidosis of the renal arteries with atheromatous plaques in the aorta, which sometimes exhibited a xanthogranulomatous structure with foreign body giant cells and giant cells of the osteoclastic type (Natali, Heine, Chester, Hess and Schildhaus)

*Diabetes Mellitus*—The disease, especially when not controlled by insulin, is often associated with a considerable increase in the cholesterol of the blood, which forms a part of the general hyperlipemia (Joslin, Warren, Hunt, Katsch and Kramick, Gibbs, Buckner and Bloor, Rabinowitch, Schondorff, Strauss, Schuetz)

Diabetic hypercholesteremia, which is not infrequently accompanied by the appearance of xanthelasma, is also associated with an increased incidence of atheromatosis affecting mainly the coronary arteries and those of the lower extremities Rabinowitch stated that the usual time necessary for the development of arteriosclerosis in diabetes is five years Dia-

betic arteriosclerosis develops within this period regardless of age (Rabinowitch, Richie and McKee) These investigators expressed the belief that an intimate relation exists between cardiovascular disease in diabetes and hypercholesteremia, which in their opinion is an important factor in the production of atherosclerosis in the young patient with diabetes Hunt noted that arteriosclerosis develops prematurely even in juvenile diabetic patients and is increasingly important as a cause of death in adult ones Similar observations were made in this respect by many others (Joslin, Warren, Gibbs, Buckner and Bloor, Schuetz, Strome and Blaine, Dublin and Lotka, Root, Bland, Gordon and White, Davis and Warren, Blotner, Strauss, White, Vander Vee, Pilgersdorfer, Lisa, Magiday, Galloway and Hart, Bell, Lisa, Magiday and Hart, Kauvar, Geiling, Dry and Hines) Dry and Hines estimated that the absolute incidence of arteriosclerosis to the degree of causing arterial insufficiency is much higher in diabetic than in nondiabetic patients (11:1) It is also known that an obliterating atherosclerotic process in the arteries of the legs which is usually more common in men than in women is much more frequent in diabetic women than in diabetic men, reversing the usual sex ratio (Dry and Hines, Collens and Wilensky) This apparent sex factor, however, may be related to the fact that diabetic women live longer than diabetic men

It may be mentioned that atherosclerotic lesions of the aorta and of the coronary arteries have been found by Fisher, Dragstedt and Hueper in dogs surviving complete pancreatectomy by several months

*Hypothyroidism*—An additional source of hypercholesteremia exists in connection with hypothyroidism (cretinism, myxedema, athyrosis), affecting young and old persons alike (Gildea, Man and Peters, Bronstein, Gilligan, Volk, Davis and Blumgart, Heckscher, Mason, Hunt and Huxthal, Gardner and Gainsborough, Greene, Hurxthal, Hurxthal and Simpson, Beutel, Fleischmann and Wilkins, Lerman, Means, Hertz and Lerman, Shelton, Wislicki, Zondek, Wilkins and Fleischmann, Wilkins, Fleischmann and Block, Thompson, Stokes, Fenz and Zell, Badwin, Michelson, Melnick and Gottfried) Similar observations were made in thyroidectomized animals (dogs, rabbits, rats) (Chaikoff, Entenman, Changus and Reichert, Fleischmann, Shumacker and Wilkins, Heckscher, Remond, Columbiés and Bernardberg, Juctschenko, Bollman and Flock, Thompson and Long, Onizawa, Messina, Fleischmann and Shumacker, Westra and Kunde, Sakurai,

Schmidt and Hughes) Craig, Lissner and Soley observed tuberous xanthomas in a case of extreme myxedematous hypercholesterolemia. Duncan asserted that the hypercholesterolemia usually present in persons with mental deterioration is the result of functional hypothyroidism caused by disuse of the thyroid gland. It may be noted in this connection that hyperfunction of the thyroid gland is associated with a low level of blood cholesterol (Schally).

Hurxthal found that the increase of serum cholesterol in hypothyroidism is not the result of lowered metabolism, as hypometabolic states of adrenal or pituitary derivation are not accompanied by hypercholesterolemia. This conclusion is supported by an observation of Page and Fari who reported that the hypercholesterolemia associated with nephrosis was not affected when thyroid medication was given and thereby the low basal metabolic rate elevated. It must be mentioned that in myxedema hypercholesterolemia and lowered basal metabolism are not the only abnormalities present. The minute volume is lowered, the circulation time is prolonged (Stewart and Evans, Wislicki), the number of erythrocytes is reduced (Thompson), the erythropoietic tissue is atrophied (Langhans), the plasma proteins and bicarbonates are increased, the excretion of calcium is decreased, the lipid phosphorus of the plasma is increased to the same extent as the cholesterol (Peters and Marx) and the distribution of cholesterol between the tissues and the plasma is shifted in favor of the plasma, while the excretion of cholesterol is unaltered (Fleischmann and Wilkins).

The relation of these hypercholesteremic changes in hypothyroidism to the development and the incidence of atherosclerosis is established by the following observations. De Coucy noted that atherosclerosis is a frequent complication of myxedema. Falta mentioned the precocious occurrence of atherosclerosis in the adult form of myxedema. Black and Kampmeier reported that the correlation between arteriosclerosis and hypothyroid states was first described as early as 1888 and recorded several cases of hypothyroidism complicated by coronary arteriosclerosis. The occurrence of large atheromas in the aorta and in other arteries in cases of aplasia of the thyroid gland has been noted (Bourneville, Maresch, Marchand, Heyn and others, Rossle, Wegelin). Merkel reported in detail such a case with extensive arterial atheromatosis and calcinosis. Mason, Hunt and Hurxthal speculated on the basis of the hypercholesterolemia observed in hypothyroidosis, stating that if arteriosclerosis is associated with abnormal retention of cholesterol together with decreased output of calcium one would expect a

high incidence of arteriosclerosis in patients with myxedema and a lowered incidence in patients with hyperthyroidism. Wegelin often noted in endemic cretinism arteriosclerosis of all degrees and called attention to the fact that atherosclerosis is frequent in those parts of Switzerland and Austria in which endemic goiter prevails. He concluded from this evidence that a severe reduction in the basal metabolic rate favors deposition of lipid and calcium. Maine described a case of myxedema in which there was generalized arteriosclerosis. Hoelzer saw extensive calcinosis preceded by lipoidosis of the muscular arteries in a 3 year old girl with congenital athyreosis. Isenschmid, as well as Wegelin, reported the occurrence of marked arteriosclerosis in cretins and attributed this to a metabolic cholesterol disturbance. Biuger and Rosenkrantz related the physiologic decrease of the thyroid activity and thus of the metabolism with age to the development of arteriosclerosis. However, as mentioned before, extensive investigations on the blood cholesterol level at various age periods have not shown any consistent elevations of this factor with advanced age.

These observations in man were confirmed by those made in animals. Von Eiselsberg obtained in goats and sheep after thyroidectomy severe aortic sclerosis and less considerable coronary arteriosclerosis. Similar observations were made by Pick and Pineles in 6 thyroidectomized goats, in which the sclerotic lesions affected the ascending part of the aorta, with calcinosis of the inner part of the media. Falta noted that hyperthyroidotic patients, on the other hand, rarely show atheromatosis, while Dungal reported that the inhabitants of Iceland, who have small, well functioning thyroid glands because of an adequate intake of iodine, show an unusually low rate of atherosclerosis of the large and small arteries.

Whereas the artificial production of hypothyroidism was accomplished in the past mainly by surgical or roentgenologic methods, recent studies have shown that this result can be obtained also by chemical means which interfere with the proper function and secretion of this gland, i. e., by prolonged medication with potassium iodide or iodotyrosine (Ghaloungui and Zell), by administration of thiourea compounds, thiobarbiturates, thiocyanides and sulfonamide compounds to man and animals and by feeding cabbage leaves or Brassica seeds containing cyanides to rabbits (Astwood, Mackenzie and Mackenzie, Mackenzie, Mackenzie and McCollum, Richter and Clisby, Kennedy, Astwood, Sullivan, Bissell and Tyslowitz, Blum, Whitehead, and many others). Detailed studies as to the effect of such medication over prolonged periods on the chole-



terol metabolism and on the vascular system are still outstanding but seem to be important in connection with aviation medicine and the therapy of hyperthyroidism

*Chronic Renal Disease*—Hypercholesteremia is a frequent hematic reaction in chronic renal disease characterized by degenerative processes of the tubules. The rise in blood cholesterol accompanying nephrosis may reach high figures (1,400 mg per hundred cubic centimeters), there is a particularly marked increase of the ester fraction as well as of phosphatides and fatty acids (Petersilie, Lowenthal, Lichtenstein and Epstein, Gainsborough, Knauer, Blackman, Gilbert Volhard, Calvin and Goldbeig, Cook). It is usually combined with severe hypoproteinemia (absolute hypalbuminemia, relative hyperglobulinemia). Lowenthal, Rosenthal and Gilbert have described the occurrence of minor to extensive atheromatosis in the aorta and coronary arteries in 6 patients with lipid nephrosis ranging in age from 4 to 29 years. Similar observations were made by others (Bing and Heckscher, Murphy and Warfield, Mason, Oppenheimer and Fishberg). Identical hematic lipid conditions prevail not infrequently in chronic glomerulonephritis with a nephrotic component. The hypercholesteremia here may also reach considerable values (Volhard, Steiner and Domanski, Epstein, Page, Kirby and Van Slyke, Lichtenstein and Epstein, Fahr, Redfield, Clark, Lauritzen, Chauffard, Laroche and Grigaut, Peters and Man, and many others), but xanthomatous manifestations are exceptional (Geyer). Inasmuch as chronic renal disease, like hypothyroidism, is often also associated with a disturbance of calcium metabolism, the atheromatous and atherosclerotic reactions observed in these conditions not only may be combined with those of the vasotonic and hydrostatic types but may sometimes show extensive intimal and medial calcification. Age factors are not involved in the production of these lesions as chronic renal diseases represent the most frequent source of arteriosclerosis in childhood (Volhard, Ophuls, Addis and Oliver). Epstein pointed out that the basal metabolic rate is often low in patients with nephritic lipemia, but there does not exist a consistent relation between these two factors. It has been found, however, that in nephrotic lipemia the degree of hypercholesteremia exhibits an inverse relation to the degree of hypoproteinemia.

*EXOGENOUS DISTURBANCES*—The effects on the vascular system of the hyperlipemia and hypercholesteremia seen after starvation as the result of the mobilization of body fat, after severe hemorrhage (Katsch, De Langen), after

introduction of barbiturates, after anesthesia, in chronic benzene poisoning in man and rabbits (Nikulín and Hetmann), in chronic thallium poisoning (Klopstock), in anemia due to phenylhydrazine and hydroxylamine (Rosenthal and Meier, De Langen, Boggs and Morris, Morawitz and Platt, Sakai) and in connection with chronic benzene poisoning in man and rabbits (Gate, Charnal, Vallet and Humbert, Grütz, Wise) are unknown. Okuneff reported only the presence of lipid material in the endothelial cells and the intima of the splenic follicular arteries of starved rabbits. It may be mentioned in this connection that certain types of chemical poisoning elicit prolonged hypercholesteremia. Thus Bang saw in dogs and particularly in rabbits an increase of the blood cholesterol after phosphorus poisoning. Sokoloff observed only a minor increase of blood cholesterol in rabbits poisoned with arsenic or phosphorus. A similar effect was elicited by injected diphtheria toxin. Strauss and Scheer reported a rise of cholesterol in the blood (up to 400 to 900 mg per hundred cubic centimeters) in connection with vascular disturbances in heavy smokers. In animals exposed to nicotine over long periods the blood cholesterol level moved in an opposite direction from the iodine level, which was elevated in some animals and lowered in others, while the thyroid gland showed in some cases a hyperplastic condition and in others an atrophic myxedematous state. These observations suggest that in chronic nicotine poisoning a cholesterologenic atheromatous factor may complicate the primary vasospastic action of the alkaloid.

*Carbon Disulfide and Hydrogen Sulfide Poisoning*—Audo-Gianotti, Bianchi and Lewey noted in viscose workers exposed occupationally to the inhalation of carbon disulfide and hydrogen sulfide vapors a type of lipemia characterized mainly by an increase of free cholesterol and phosphatides. It is not known whether or not this lipemic reaction is of cytolytic origin and attributable to the destruction of nervous substance of the brain commonly seen in poisoning of this type. The workers had in addition to mental and neurogenic manifestations an unusually high incidence of cardiovascular disturbances, including angina pectoris (Gallego, Gordy and Trumper, Lewey). Audo-Gianotti noted the presence of vascular changes in viscose workers, which he attributed to the action of the "sulfmethemoglobin" formed. Arteriosclerotic lesions of the peripheral and retinal arteries of such patients were reported by Lewey and McDonald. The cerebral arteries of man, dogs and cats poisoned with carbon disulfide were observed to contain marked sclerotic changes (Lewey,

Alpers, Ferrari, Jarvis and Flicker) Lewey attributed the vascular lesions to the increased incidence of hypertension among young viscose workers exposed to carbon disulfide and discounted any causal role of the hypercholesterolemia in this respect

**Saponin Poisoning**—The parenteral introduction of saponins results in the development of an apparently cytolytic type of hypercholesterolemia lasting up to twelve days after the injection (Kollet and Grill, Kollert, Kofler and Susan, Kollet and Rezek, Handovsky, Handovsky and von Trossel Lasch, Ehrenteil and Weiss-Ostborn) Saponins cause, moreover, a disturbance in the colloidal lipid equilibrium of the plasma which influences the molecular dispersion of cholesterol (Ehrentheil and Weiss-Ostborn) and thereby the colloidal stability of the plasma (Lasch, Sobotka) This effect is due in part to the binding of cholesterol by saponin (Wacker and Hueck, Kofler and Fischer, Bayer and Gaisbock) Pachonukow reported the presence of jelly-like or fibrous nodular deposits firmly attached to the cardiac valvular leaflets (saponin endocarditis of Kobert) in animals given intravenous injections of saponin Handovsky attributed the development of "arteriosclerotic"

cholesterol deposits in the aortic intima of rabbits given saponin intravenously to the altered distribution of cholesterol in the plasma

**Physical Labor**—Severe and persistent physical labor elicits a hypercholesteremic reaction (Knack, Picard) Wacker and Hueck suggested that the prevalence of atherosclerosis in males might be attributable to this factor It is doubtful, however, whether the manual labor of the male accounts to any appreciable degree for the definite difference in morbidity and mortality from atherosclerosis between the two sexes (Leary) It is more probable that a factor related to a sex hormone plays a role in this respect by preventing the deposition of cholesterol in the arterial walls The existence of such relations is suggested by the fact that the hypercholesteremia accompanying pregnancy (Gruenfeld and Seelig, Katsch, Saito, Gardner) does not favor the development of atherosclerotic lesions but leads merely to the appearance of transitory lipid spots (pregnancy spots) in the aortic intima (Popken) After parturition the cholesterol level of the maternal blood drops sharply, the excess cholesterol being apparently excreted with the milk (Knaue)

(To Be Concluded)

## Notes and News

**Grants in Aid**—Applications to the Committee for Research in Problems of Sex of the National Research Council for financial aid during the fiscal year beginning on July 1, in support of work on fundamental problems of sex and reproduction, should be received before April 1 They may be addressed to the chairman, Dr Robert M Yerkes, Yale School of Medicine, New Haven 11, Conn Although hormonal investigations continue to command the interest and support of the committee, preference, in accordance with current policy, will ordinarily be given to proposals for an investigation of neurologic, psychobiologic and behavioral problems of sex and reproduction

The Ella Sachs Plotz Foundation announces that grants will be given in aid of research in the sciences closely related to medicine As a rule, the maximum amount granted is less than \$500 Applications for grants to be held during the year 1945-1946 must be in the hands of the executive committee before April 1945 They should be sent to Dr Joseph C Aub, Massachusetts General Hospital, Fruit Street, Boston 14 There are no formal application blanks, but letters asking for aid must describe accurately the qualifications of the investigator and the research, name the amount requested and state whether other requests for support have been made

**Appointment**—Daniel Fowler Cappel, professor of pathology at the University of St Andrews, has been appointed to the chair of pathology at the University of Glasgow, Scotland, in succession to the late Shaw Dunn

**Deaths**—Sir Joseph Arkwright, long connected with the Lister Institute, London, England, died Nov 22, 1944, in his eighty-first year He made important contributions to the knowledge of carriers of infectious diseases and to that of trench fever and typhus, of bacterial variation and of certain animal diseases

**Society News**—The annual meeting of the American Association for Cancer Research will be held at Cleveland, Monday and Tuesday, May 7 and 8, 1945, preceding the meetings of the Federation of American Societies for Experimental Biology Dr Lawrence A Pomeroy, 952 Hanna Building, Cleveland, is in charge of arrangements It is tentatively announced that the Hotel Cleveland will be the headquarters

The Society of American Bacteriologists will meet at the Book-Cadillac Hotel, Detroit, May 22 to 25, 1945, under the presidency of I L Baldwin The secretary is L W Parr, Washington University, Washington D C

The Federation of American Societies for Experimental Biology will meet in Cleveland, May 7 to 10 inclusive, 1945



The Toledo Hospital Institute of Medical Research, Toledo, Ohio, has received a grant of \$13,000 from the Snyder Ophthalmic Foundation for research on the physiology of the eye especially in relation to glaucoma

The grant will be extended depending on the results obtained in the first two years. Because of the war, research will be delayed until a physiologist with specialized training in the eye become available

## Correspondence

### IMPORTANCE OF TITLES THAT ACCURATELY REFLECT THE SUBJECTS OF THE ARTICLES

*To the Editor* —In the December 1944 issue of the ARCHIVES OF PATHOLOGY there appeared an article by Albert E. Casey and Eleanor H. Hidden entitled "Non-development of the Septum Transversum," in which they described a major defect in the diaphragm of a newborn infant who lived only four hours.

Autopsy showed absence of the central tendon and anterior portion of the diaphragm with the presence of a *pericardioperitoneal* foramen.

In the summary given in this paper it is stated "This is the first instance of this combination of anomalies to be recorded in the literature." They offer the case as proof of the embryologic concept that the septum transversum is "the anlage of the central tendon of the diaphragm, the ventrocaudal portion of the pericardium and the suspensory ligament of the liver."

Further, in the comment they mention Giffin's<sup>1</sup> review of the literature on diaphragmatic hernia (1912), in which he said that herniation into the pericardial sac had been reported but that he was unable to find the original description of the case and so could not state whether there was a direct or an indirect communication between the pericardial and the peritoneal cavity.

Casey and Hidden also refer to three papers by Hedbloom (1925, 1931 and 1934). In Hedbloom's first paper,<sup>2</sup> which contains an analysis of 378 cases in which diaphragmatic hernia was treated surgically, only 1 case is listed as a case of pericardial hernia, but it is not described nor is a reference given. In his last two papers Hedbloom made no note of pericardial hernia, and a personal communication from Hedbloom to Casey and Hidden indicated that he had not encountered a hernia extending into the pericardial sac in more than 400 cases in which he had operated for diaphragmatic hernia.

Finally, after a personal communication from Giffin, Casey and Hidden state that "Other than Giffin's and Hedbloom's mention of a diaphragmatic hernia extending into the pericardial sac no references to such an anomaly could be found in the literature. Since Hedbloom in his final report makes no reference to diaphragmatic herniation into the pericardial sac, his original mention probably referred to the defect cited by Giffin. Giffin has in his notes no reference to a direct communication between the pericardial and peritoneal cavities and states that since an original article describing such a case cannot be found, we are

warranted in considering ours [Casey and Hidden] the first to be reported."

In order to set the records straight, I wish to state that in 1909 I reported a case in which there was a direct communication between the peritoneal and pericardial cavities and herniation of the transverse colon and great omentum into the pericardial sac.<sup>3</sup> This case, published under the title "A Case of Congenital False Diaphragmatic Hernia," is probably the one that Giffin had not been able to find.

The defect in this case was attributed to arrested development of the septum transversum and seemed to be of the nature of a teratologic rather than an embryologic fault and one closely allied to a beginning subthoracic ectopia cordis.

This anomaly occurred in a white man, aged 70, who had never had any serious illness throughout his life and had suffered no traumatism to his chest or abdomen. He was admitted to the hospital markedly cyanotic and dyspneic and lived seventeen hours. The clinical diagnosis was congestive heart failure.

Autopsy showed a large defect in the central portion of the diaphragm, 6 cm. in diameter, through which the hand could be passed directly into the pericardial cavity. The distended transverse colon and most of the great omentum was virtually wrapped about the heart, causing an asphyxial type of death. Chronic adhesive peritonitis over several loops of small intestine suggested that such portions had at times herniated into the pericardial sac. There were no other anomalies.

I reported the case on account of its great rarity, as I was unable to find mention of any similar case in the literature. It was also of interest because of the age which the man reached with such a major defect.

Since this report I have never come across any reference to any similar case in the many articles which have appeared on diaphragmatic hernia.

The purpose of this communication is to emphasize the importance of the careful selection of titles for all medical articles. Many important papers and original observations are virtually lost in the enormous literature because the titles are not sufficiently descriptive. For instance, the title of my paper, "A Case of Congenital False Diaphragmatic Hernia," gives no indication of the presence of herniation into the pericardial cavity, which was the main reason for publication. With such a title it is impossible to give a paper proper classification in an index. Thus under the title quoted the article would be regarded by many workers as just another report of the ordinary, commonly recognized diaphragmatic hernia.

HARRISON S. MARTLAND, M.D., Newark, N. J.

1 Giffin, H. Z. Ann Surg 55 388, 1912

2 Hedbloom, C. A. J. A. M. A. 85 947, 1925

3 Martland, H. S. Paper read at meeting of the New York Pathological Society, Jan 13, 1909, J. A. M. A. 52 1574, 1909

## EXPERIMENTAL NEPHROPATHIES

### II RENAL PHOSPHATASE AFTER POISONING WITH MERCURY BICHLORIDE, URANYL NITRATE AND POTASSIUM DICHROMATE

OPAL E HEPLER, MD, HELEN GURLEY, MS, AND  
JAMES P SIMONDS, MD

CHICAGO

Phosphatase is an enzyme, first discovered in the mucosa of the duodenum by Grosser and Husler<sup>1</sup> in 1912, which splits the  $\text{PO}_4$  ion from the esters of phosphoric acid. Two types have been differentiated, "alkaline," which is most active in a medium with  $p_{\text{H}}$  about 9.0, and "acid," which acts best in a medium with  $p_{\text{H}}$  between 5.0 and 6.0. Both types can be readily demonstrated in tissues by microtechnical (Gomori<sup>2</sup>) and chemical (Bodansky<sup>3</sup>) methods. This enzyme is present in blood serum (Bodansky<sup>3</sup>, Thannhauser and co-workers<sup>4</sup>), in bile (Freeman, Chen and Ivy<sup>5</sup>), in urine (Kutscher and Wolbergs<sup>6</sup>) and in human semen (Huggins and Johnson<sup>7</sup>).

The pattern of the distribution of phosphatase in the different organs of animals belonging to the same species is relatively constant (Gomori<sup>2a</sup>). Even in animals of different species the location of the enzyme in corresponding organs is much more constant than is its amount. Thus, MacFarlane, Patterson and Robinson,<sup>8</sup> using chemical methods, found increasing quantities of alkaline renal phosphatase in the following order of the species examined: rabbit, rat, guinea pig,

dog, cat and mouse. Not only did the kidneys of the dog, the cat and the mouse contain more of the enzyme than did those of the rabbit, the rat and the guinea pig, but the renal phosphatase of the former group was activated to a much greater degree by magnesium. This observation is important in the selection of animals in which to study the phosphatase of the kidneys. Wherever found, alkaline phosphatase, stained by Gomori's method, is granular in appearance. In the kidneys it is limited to the brush border of the epithelium of the proximal convoluted tubules (Gomori<sup>2a</sup>, Hepler, Gurley and Simonds<sup>9</sup>). Elsewhere it is distributed through the cytoplasm of the cells. In the small intestine it is limited to the superficial epithelial cells of the mucosa.

Our experiments were undertaken with the hope of learning something concerning either the function or the mechanism of action of alkaline renal phosphatase. In another paper two of us<sup>10</sup> have presented evidence, confirming earlier work by Suzuki,<sup>11</sup> that in dogs potassium dichromate injures chiefly the first part, and mercury bichloride and uranyl nitrate chiefly the terminal portion, of the proximal convoluted tubule. In previous experiments we had determined the smallest dose of each of these poisons that would produce detectable injury of the renal tubules without visibly affecting the glomeruli. It appeared possible, therefore, that by injecting optimal doses directly into the blood stream varying degrees of damage up to complete necrosis might be inflicted on different parts of the proximal convoluted tubule and thus permit a study of these effects on the phosphatase activity of the kidneys.

From the Department of Pathology, Northwestern University Medical School.

This investigation was aided by a grant from the Committee on Experimental Therapeutics of the American Medical Association.

1 Grosser, P., and Husler, J. *Biochem Ztschr* **39** 1, 1912.

2 Gomori, G. (a) *Proc Soc Exper Biol & Med* **42** 23, 1939, (b) *J Cell & Comp Physiol* **17** 71, 1941, *Arch Path* **32** 189, 1941.

3 Bodansky, A. *J Biol Chem* **101** 93, 1933.

4 Thannhauser, S. J., Reichel, M., and Frattani, J. F. *J Biol Chem* **121** 697, 1937.

5 Freeman, S., Chen, Y. P., and Ivy, A. C. *J Biol Chem* **124** 79, 1938.

6 Kutscher, W., and Wolbergs, H. *Naturwissenschaften* **23** 559, 1935.

7 Huggins, C., and Johnson, A. A. *Am J Physiol* **103** 574, 1933.

8 MacFarlane, M. G., Patterson, L. M. B., and Robison, R. *Biochem J* **281** 720, 1934.

9 Hepler, O. E., Gurley, H., and Simonds, J. P. *Proc Soc Exper Biol & Med* **44** 221, 1940.

10 Simonds, J. P., and Hepler, O. E. *Arch Path* **39** 103, 1945.

11 Suzuki, T. *Morphologie der Nierensekretion unter physiologischen und pathologischen Bedingungen*, Jena, G. Fischer, 1912.

The Toledo Hospital Institute of Medical Research, Toledo, Ohio, has received a grant of \$13,000 from the Snyder Ophthalmic Foundation for research on the physiology of the eye especially in relation to glaucoma

The grant will be extended depending on the results obtained in the first two years. Because of the war, research will be delayed until a physiologist with specialized training in the eye become available

## Correspondence

### IMPORTANCE OF TITLES THAT ACCURATELY REFLECT THE SUBJECTS OF THE ARTICLES

*To the Editor*—In the December 1944 issue of the ARCHIVES OF PATHOLOGY there appeared an article by Albert E Casey and Eleanor H Hidden entitled "Non-development of the Septum Transversum," in which they described a major defect in the diaphragm of a newborn infant who lived only four hours

Autopsy showed absence of the central tendon and anterior portion of the diaphragm with the presence of a *pericardioperitoneal* foramen

In the summary given in this paper it is stated "This is the first instance of this combination of anomalies to be recorded in the literature" They offer the case as proof of the embryologic concept that the septum transversum is "the anlage of the central tendon of the diaphragm, the ventrocaudal portion of the pericardium and the suspensory ligament of the liver"

Further, in the comment they mention Giffin's<sup>1</sup> review of the literature on diaphragmatic hernia (1912), in which he said that herniation into the pericardial sac had been reported but that he was unable to find the original description of the case and so could not state whether there was a direct or an indirect communication between the pericardial and the peritoneal cavity

Casey and Hidden also refer to three papers by Hedbloom (1925, 1931 and 1934) In Hedbloom's first paper,<sup>2</sup> which contains an analysis of 378 cases in which diaphragmatic hernia was treated surgically, only 1 case is listed as a case of pericardial hernia, but it is not described nor is a reference given In his last two papers Hedbloom made no note of pericardial hernia, and a personal communication from Hedbloom to Casey and Hidden indicated that he had not encountered a hernia extending into the pericardial sac in more than 400 cases in which he had operated for diaphragmatic hernia

Finally, after a personal communication from Giffin, Casey and Hidden state that "Other than Giffin's and Hedbloom's mention of a diaphragmatic hernia extending into the pericardial sac no references to such an anomaly could be found in the literature Since Hedbloom in his final report makes no reference to diaphragmatic herniation into the pericardial sac, his original mention probably referred to the defect cited by Giffin Giffin has in his notes no reference to a direct communication between the pericardial and peritoneal cavities and states that since an original article describing such a case cannot be found, we are

warranted in considering ours [Casey and Hidden] the first to be reported"

In order to set the records straight, I wish to state that in 1909 I reported a case in which there was a direct communication between the peritoneal and pericardial cavities and herniation of the transverse colon and great omentum into the pericardial sac<sup>3</sup> This case, published under the title "A Case of Congenital False Diaphragmatic Hernia," is probably the one that Giffin had not been able to find

The defect in this case was attributed to arrested development of the septum transversum and seemed to be of the nature of a teratologic rather than an embryologic fault and one closely allied to a beginning sub-thoracic ectopia cordis

This anomaly occurred in a white man, aged 70, who had never had any serious illness throughout his life and had suffered no traumatism to his chest or abdomen He was admitted to the hospital markedly cyanotic and dyspneic and lived seventeen hours The clinical diagnosis was congestive heart failure

Autopsy showed a large defect in the central portion of the diaphragm, 6 cm in diameter, through which the hand could be passed directly into the pericardial cavity The distended transverse colon and most of the great omentum was virtually wrapped about the heart, causing an asphyxial type of death Chronic adhesive peritonitis over several loops of small intestine suggested that such portions had at times herniated into the pericardial sac There were no other anomalies

I reported the case on account of its great rarity, as I was unable to find mention of any similar case in the literature It was also of interest because of the age which the man reached with such a major defect

Since this report I have never come across any reference to any similar case in the many articles which have appeared on diaphragmatic hernia

The purpose of this communication is to emphasize the importance of the careful selection of titles for all medical articles Many important papers and original observations are virtually lost in the enormous literature because the titles are not sufficiently descriptive For instance, the title of my paper, "A Case of Congenital False Diaphragmatic Hernia," gives no indication of the presence of herniation into the pericardial cavity, which was the main reason for publication With such a title it is impossible to give a paper proper classification in an index Thus under the title quoted the article would be regarded by many workers as just another report of the ordinary, commonly recognized diaphragmatic hernia

HARRISON S MARTLAND, M D, Newark, N J

1 Giffin, H Z Ann Surg 55 388, 1912

2 Hedbloom, C A J A M A 85 947, 1925

3 Martland, H S Paper read at meeting of the New York Pathological Society, Jan 13, 1909, J A M A 52 1574, 1909

## EXPERIMENTAL NEPHROPATHIES

### II RENAL PHOSPHATASE AFTER POISONING WITH MERCURY BICHLORIDE, URANYL NITRATE AND POTASSIUM DICHROMATE

OPAL E HEPLER, MD, HELEN GURLEY, MS, AND  
JAMES P SIMONDS, MD  
CHICAGO

Phosphatase is an enzyme first discovered in the mucosa of the duodenum by Grosse and Husler<sup>1</sup> in 1912, which splits the  $\text{PO}_4$  ion from the esters of phosphoric acid. Two types have been differentiated, "alkaline," which is most active in a medium with  $p_{\text{H}}$  about 9.0, and "acid," which acts best in a medium with  $p_{\text{H}}$  between 5.0 and 6.0. Both types can be readily demonstrated in tissues by microtechnical (Gomori<sup>2</sup>) and chemical (Bodansky<sup>3</sup>) methods. This enzyme is present in blood serum (Bodansky<sup>3</sup>, Thannhauser and co-workers<sup>4</sup>), in bile (Freeman, Chen and Ivy<sup>5</sup>), in urine (Kutscher and Wolbergs<sup>6</sup>) and in human semen (Huggins and Johnson<sup>7</sup>).

The pattern of the distribution of phosphatase in the different organs of animals belonging to the same species is relatively constant (Gomori<sup>2a</sup>). Even in animals of different species the location of the enzyme in corresponding organs is much more constant than is its amount. Thus, MacFarlane, Patterson and Robinson,<sup>8</sup> using chemical methods, found increasing quantities of alkaline renal phosphatase in the following order of the species examined: rabbit, rat, guinea pig,

dog, cat and mouse. Not only did the kidneys of the dog, the cat and the mouse contain more of the enzyme than did those of the rabbit, the rat and the guinea pig, but the renal phosphatase of the former group was activated to a much greater degree by magnesium. This observation is important in the selection of animals in which to study the phosphatase of the kidneys. Wherever found, alkaline phosphatase, stained by Gomori's method, is granular in appearance. In the kidneys it is limited to the brush border of the epithelium of the proximal convoluted tubules (Gomori<sup>2a</sup>, Hepler, Gurley and Simonds<sup>9</sup>). Elsewhere it is distributed through the cytoplasm of the cells. In the small intestine it is limited to the superficial epithelial cells of the mucosa.

Our experiments were undertaken with the hope of learning something concerning either the function or the mechanism of action of alkaline renal phosphatase. In another paper two of us<sup>10</sup> have presented evidence, confirming earlier work by Suzuki,<sup>11</sup> that in dogs potassium dichromate injures chiefly the first part, and mercury bichloride and uranyl nitrate chiefly the terminal portion, of the proximal convoluted tubule. In previous experiments we had determined the smallest dose of each of these poisons that would produce detectable injury of the renal tubules without visibly affecting the glomeruli. It appeared possible, therefore, that by injecting optimal doses directly into the blood stream varying degrees of damage up to complete necrosis might be inflicted on different parts of the proximal convoluted tubule and thus permit a study of these effects on the phosphatase activity of the kidneys.

From the Department of Pathology, Northwestern University Medical School.

This investigation was aided by a grant from the Committee on Experimental Therapeutics of the American Medical Association.

1 Grosse, P., and Husler, J. *Biochem Ztschr* **39** 1, 1912.

2 Gomori, G. (a) *Proc Soc Exper Biol & Med* **42** 23, 1939, (b) *J Cell & Comp Physiol* **17** 71, 1941, *Arch Path* **32** 189, 1941.

3 Bodansky, A. *J Biol Chem* **101** 93, 1933.

4 Thannhauser, S. J., Reichel, M., and Frattin, J. F. *J Biol Chem* **121** 697, 1937.

5 Freeman, S., Chen, Y. P., and Ivy, A. C. *J Biol Chem* **124** 79, 1938.

6 Kutscher, W., and Wolbergs, H. *Naturwissenschaften* **23** 559, 1935.

7 Huggins, C., and Johnson, A. A. *Am J Physiol* **103** 574, 1933.

8 MacFarlane, M. G., Patterson, L. M. B., and Robison, R. *Biochem J* **281** 720, 1934.

9 Hepler, O. E., Gurley, H., and Simonds, J. P. *Proc Soc Exper Biol & Med* **44** 221, 1940.

10 Simonds, J. P., and Hepler, O. E. *Arch Path* **39** 103, 1945.

11 Suzuki, T. *Morphologie der Nierensekretion unter physiologischen und pathologischen Bedingungen*, Jena, G. Fischer, 1912.

METHODS

All our experiments were made on dogs, and the methods used are described in a previous paper<sup>10</sup> One group of dogs was poisoned with mercury bichloride, a second group with potassium dichromate and a third with uranyl nitrate The total volume of circulating blood was estimated in each animal by the formula  $\text{Weight in grams} \times 0.0925 = \text{volume of blood in cubic centimeters}$  The poison was dissolved in isotonic solution of sodium chloride and injected slowly into a vein in such amount that the dog received one or more doses of 0.1 to 3 mg of the poison for each 100 cc of circulating blood These dogs either died or were killed from twenty-eight hours to eight days after the first injection of the poison The phosphatase of the serum and that of the renal cortex were determined by Bodansky's<sup>3</sup> method Sections of the kidneys stained by Gomori's<sup>2a</sup> technic were employed in order to demonstrate any deviation from the normal in the amount, the form or the distribution of phosphatase in the tubular epithelium

PHOSPHATASE IN NORMAL CONTROLS

Chemical determinations were made of the phosphatase in the blood serum of 13, and in the renal cortex of 37, unselected normal dogs, some of which had been subjected to acute experiments of various kinds in the department of physiology of Northwestern University Medical School The results of these determinations are shown in table 1

TABLE 1—*Determinations of Renal and Serum Phosphatase in Normal Dogs*

	Dogs	Mean in Bodansky Units	Standard Deviation
Serum phosphatase	13	$3.69 \pm 0.32^*$	$0.55 \pm 0.23$
Renal phosphatase	37	$10.72 \pm 0.33^\dagger$	$2.99 \pm 0.23$

\* Per hundred cubic centimeters  
† Per gram

The range of serum phosphatase was relatively narrow, as is shown by the comparatively small standard deviation, that of renal phosphatase was much wider, its standard deviation being about one fourth of the mean value In some tissues, notably in bone, the amount of phosphatase is known to vary inversely with the age of the animal No results appear to be available that would indicate whether this is true of the kidneys Furthermore, prolonged ether anesthesia probably reduces the phosphatase activity of the kidneys Since some of these animals had been subjected to such anesthesia for varying periods, this may have been a factor in the wide range of the number of phosphatase units for the different dogs in this series

In sections of normal kidneys stained by Gomori's method the phosphatase, being sharply limited to the brush border of the epithelium, forms a narrow ring about the lumen of the proximal convoluted tubule This characteristic

distribution may be inherently related to the fact that it is the function of the epithelium of the proximal convoluted tubules to absorb the contents of the lumens and not to produce some substance and excrete it into the lumens

PHOSPHATASE IN DOGS POISONED WITH MERCURY BICHLORIDE

Dogs were given mercury bichloride in the manner described under "Methods" The number and the size of the doses were varied for different animals so that they received one or more injections of 1, 2 or 3 mg, respectively, of mercury bichloride for each 100 cc of blood These animals died or were killed from twenty-eight hours to eight days after the first injection of the poison The phosphatase of the serum was determined before, and from one to four times after, the injection, and the results in dogs with at least three determinations are shown in table 2

TABLE 2—*Determinations of Serum Phosphatase in Twelve Dogs Poisoned with Mercury Bichloride*

	Bodansky Units
Average of values before injection	3.00
Average of minimum values after injection	2.75
Average of maximum values after injection	4.75
Average of final values after injection	3.93

Since the averages in table 2 were obtained from a group of dogs not identical with that used in compiling table 1, there is a difference in the mean values of the controls in the two tables The averages of the minimum and maximum values were obtained from the lowest and highest results, respectively, in the multiple determinations after administration of the poison The average of the final values was calculated from the last determinations immediately before the animals were put to death The average of the maximum values was about 58 per cent higher, that of the final values about 33 per cent higher and that of the minimum values less than 10 per cent lower than the average of the control values of the same animals before the injection of mercury bichloride Poisoning of these dogs with this substance therefore produced definite alterations in the serum phosphatase during a period of at least eight days after the administration of the poison

Changes in the phosphatase activity of the renal cortex are influenced by the amount of poison administered, as shown in table 3

Of the 17 dogs used for the determinations in table 3, 6 died and 11 were killed five to eight days after the first injection of the poison Of 6 dogs that were given in a single dose 3 mg of mercury bichloride for each 100 cc of blood, 5

died, and the remaining animal was killed five days after the administration of the bichloride. The kidneys of the 6 dogs in this group (table 4) contained an average of 7.62 Bodansky units of phosphatase per gram, which is 29 per cent below the normal mean. The dog that survived for five days had the highest renal phosphatase (11.31 units) in this group. The average of the values for renal phosphatase in 4 dogs that were given three injections of 1 mg each of mercury bichloride for each 100 cc of blood on successive days was practically normal (10.78 units), compared with the normal mean of 10.72 units. The average of the values for renal phosphatase activity in 6 dogs that received 2

TABLE 3—*Determinations of Renal Phosphatase in Dogs Poisoned with Mercury Bichloride Showing Influence of Amount of Dose*

Dosage of HgCl <sub>2</sub>	Dogs	Renal Phosphatase	
		Mean in Bodansky Units	Standard Deviation
3 mg to 100 cc of blood	10	9.10 ± 0.75	3.50 ± 0.53
2 mg or less to 100 cc of blood	7	16.57 ± 1.39	5.44 ± 0.97

TABLE 4—*Determinations of Renal Phosphatase Showing Influence of Number of Injections of the Poison*

Dosage of HgCl <sub>2</sub> Mg	Injections	Dogs	Average of Values in Bodansky Units	Range of Values in Bodansky Units
3	1	6	7.62	4.28 to 11.31
1	3	4	10.78	7.66 to 16.90
2	1	2	16.05	6.30 to 12.90 to 22.90
1	2	4	16.05	
1	1	1	19.05	

mg of mercury bichloride for each 100 cc of blood, either in single or broken doses, was approximately 50 per cent above the normal mean. One dog in this group died on the seventh day after injection of the poison, and its kidney had the lowest content of phosphatase (6.3 units) in the group. The kidneys of a dog that was given 1 mg of mercury bichloride for each 100 cc of blood yielded 19.05 units of phosphatase (77 per cent above the normal mean). The kidneys of another dog that received a similar dose gave such extremely high phosphatase values (27.12 units, more than two and a half times the normal mean) that it was not included in any of the calculations for this whole group of dogs.

The results of the determination of the phosphatase activity of the kidneys of this series of dogs by chemical means may be briefly summarized as follows. 1. A single dose of 3 mg of mercury bichloride for each 100 cc of blood was fatal to approximately 90 per cent of the animals within three days. 2. Such fatal doses produced

extensive necrosis of the epithelium of the proximal convoluted tubules and materially reduced the phosphatase of the kidneys but did not completely destroy or inactivate the enzyme. 3. Smaller nonfatal doses of 2 mg or less of mercury bichloride for each 100 cc of blood seemed to stimulate the activity of the phosphatase of the kidneys, particularly when the dose was so small that microscopic examination revealed no actual necrosis of the tubular epithelium.

The results of the quantitative determinations of phosphatase in the kidneys of dogs poisoned with mercury bichloride were compared with sections of the same kidneys stained by Gomori's method. In normal canine kidneys the phosphatase is distributed in the brush border throughout the entire length of the proximal convoluted tubules, while the remainder of the cytoplasm of these cells is free. In the kidneys of dogs that received the minimum necrotizing dose of mercury bichloride the necrosis was limited to the distal half of the proximal convoluted tubules, particularly in their straight terminal portions, which lie in the outer part of the labyrinth along the medullary rays. The same kidneys showed no necrosis and little or no alteration in the intracellular distribution of phosphatase in the narrow aglomerular zone immediately beneath the renal capsule.<sup>10</sup> The earliest visible effect of necrotizing doses of mercury bichloride appears to be diffusion of the phosphatase throughout the cytoplasm. As long as the necrotic cell retains its form its cytoplasm is diffusely stained by Gomori's method, but more palely than is the brush border of normal cells. The results of chemical analysis indicate that the pale staining is due to actual diminution in phosphatase activity and not merely to dilution by diffusion throughout the cell. In normal canine kidneys the lumens of the tubules do not contain detectable phosphatase. When cells of the proximal convoluted tubules disintegrate after damage by mercury bichloride, the detritus in the tubular lumens gives a positive phosphatase reaction by Gomori's method.

Figures 1a and 1b show serial sections from the kidney of a dog (K Hg 6) that received a single injection of 3 mg of mercury bichloride for each 100 cc of blood and died on the third day thereafter. In section a, stained with hematoxylin and eosin, the nuclei of the epithelium of the tubules near the glomeruli, i.e., in the labyrinth, stain well while the cells of the straight terminal portions of the proximal convoluted tubules along the margins of the labyrinth are completely necrotic. In section b, stained by Gomori's method, the phosphatase in the epithelium within the labyrinth stains deeply and is in its normal position about the lumen. The phosphatase in the necrotic cells

is diminished in amount and is uniformly distributed throughout the necrotic mass. This dog's kidneys contained 11.06 units of phosphatase per gram of tissue.

PHOSPHATASE IN DOGS POISONED WITH URANYL NITRATE

The phosphatase of the blood and the kidneys of 15 dogs poisoned with uranyl nitrate was studied with the technic that was used in the study of the group poisoned with mercury bichloride. The effects are shown in table 5. One milligram or less of uranyl nitrate had little effect on blood phosphatase but increased renal phosphatase 30 per cent above the normal mean. On the other hand, doses of 2 mg. or more increased

TABLE 5—*Determinations of Phosphatase in Normal Dogs and in Dogs Poisoned with Uranyl Nitrate*

	Average of Values in Bodansky Units			
	Before Poisoning	After Injection of 1 Mg. or Less of Uranyl Nitrate	Before Poisoning	After Injection of 2 Mg. or More of Uranyl Nitrate
Blood phosphatase	2.65 (?)	3.47	3.70	5.66
Renal phosphatase		14.09		16.29

blood phosphatase 53 per cent and renal phosphatase 52 per cent above the corresponding normal means.

Staining by Gomori's method revealed phosphatase diffusely spread throughout the cytoplasm of renal cells severely damaged by uranyl nitrate and phosphatase-containing detritus in the tubules, similar to that in mercury bichloride poisoning. With minimum necrotizing doses the distribution of these changes was also similar to that in the kidneys of dogs poisoned with mercury bichloride, i. e., the necrosis of the epithelium and the alteration in the enzyme were most marked in the distal portions of the proximal convoluted tubules. In regions of complete necrosis the phosphatase stained diffusely and sometimes deeply in the necrotic mass. Breedis, Flory and Furth<sup>12</sup> described this change as "phosphatase-rich casts."

Figures 2a and 2b are from the kidney of a dog (K Ur 22) that received two injections, on successive days, of 3 mg. of uranyl nitrate for each 100 cc. of blood and was put to death on the fourth day after the first injection. This amount was far above the minimum necrotizing dose. In figure 2a, showing a section stained with hematoxylin and eosin, the epithelium of the

tubules within the labyrinth is swollen and ragged but the nuclei stain, while the straight terminal portions along the margins of the labyrinth are necrotic. In figure 2b, showing a section stained by Gomori's method, the phosphatase of the epithelium within the labyrinth stains well and is concentrated about the lumen but with slight diffusion through the cytoplasm, while the straight portion is necrotic and stains poorly but uniformly. In the left upper quadrant, beginning just below the glomerulus in the top center, is a tubule cut longitudinally through the origin of the straight terminal segment. In the upper end of this tubule the phosphatase is concentrated about the lumen but is moderately diffused through the cytoplasm. In the next short segment, where the tubule curves downward, cell outlines are lost and the entire mass is stained deeply, showing a high content of phosphatase. In the terminal straight portion of this tubule the epithelium is completely necrotic and stains palely but somewhat irregularly. In the right lower corner is a glomerulus with phosphatase-containing debris in its capsular space. This debris was probably regurgitated into the capsular space from necrotic material in more distal parts of the tubule, since the phosphatase in the epithelium in the beginning of the tubule, seen leaving the glomerulus, is almost normal in its distribution within the cells. The kidneys of this dog contained 21.70 units of phosphatase per gram of tissue.

PHOSPHATASE IN DOGS POISONED WITH POTASSIUM DICHROMATE

Studies of phosphatase were made on 9 dogs poisoned with potassium dichromate with the following results. In 5 dogs receiving 2 mg. or less for each 100 cc. of blood the renal phosphatase averaged 15.85 units per gram (47.85 per cent above the normal mean). In each of the poisoned animals the renal phosphatase was higher than the normal mean, with a range of 11.18 to 18.86 units. Four dogs received 4 to 6 mg. of potassium dichromate for each 100 cc. of blood in two doses of 2 and 3 mg. each. In this group the renal phosphatase values averaged 7.41 units (44.66 per cent below the normal mean), with a range of 6.0 to 9.6 units.

In sections of kidneys with minimal necrosis stained for phosphatase by Gomori's method the phosphatase in the terminal straight portions of the proximal convoluted tubules was limited to the brush border of the cells as in the normal controls. In the upper parts of the tubules, particularly in the subcapsular zone, and throughout the entire length of the proximal convoluted tubules

12 Breedis, C., Flory, C. M., and Furth, J. Arch Path 36: 402, 1943.



in dogs poisoned with larger doses, cell outlines were lost and the coagulated mass was stained uniformly black or pale brown

The sections in figures 3a and 3b are from the subcapsular zone of a kidney of a dog (K Cr 16) that received two injections, on successive days, of 3 mg each of potassium dichromate for each 100 cc of blood. In the first section, stained with hematoxylin and eosin, there is massive necrosis of the epithelium of the first parts of the proximal convoluted tubules. Since the amount given this dog was greatly in excess of the minimum necrotizing dose, the necrosis involved practically the entire tubule and was not limited to the first segment. In the second section, stained by Gomori's method, the necrotic material of most of the tubules stains uniformly black, which indicates that active phosphatase is still present. The kidneys of this dog contained only 6.65 units of phosphatase per gram of tissue.

#### COMMENT

The results of the experiments described in detail in the foregoing pages are summarized in table 6. In animals poisoned with small doses of mercury bichloride and potassium dichromate, i. e. 2 mg or less for each 100 cc of blood, the

TABLE 6—Summary of Determinations of Phosphatase

Phosphatase	Ratio Between Mean Values for Normal and Poisoned Dogs					
	HgCl <sub>2</sub>		UO <sub>2</sub> (NO <sub>3</sub> ) <sub>2</sub>		K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub>	
	2 Mg or Less	3 Mg	2 Mg or Less	3 Mg	2 Mg or Less	3 Mg or More
Blood						
Renal	1.154	1.085	1.094	1.1	1.069	1.022
			1.130	1.152	1.145	1.069

activity of renal phosphatase was increased on the average above the normal mean (1.154 and 1.145, respectively) to an extent that appears not to be merely the result of chance, larger doses (3 mg or more) caused marked reduction (1.085 and 1.069, respectively). This reduction was greater with potassium dichromate than with mercury bichloride, but the doses of potassium dichromate in some dogs reached a total of 6 mg per hundred cubic centimeters of blood. The mean values of renal phosphatase in both groups poisoned with uranyl nitrate, i. e., those receiving 2 mg or less and those receiving 3 mg or more per hundred cubic centimeters of blood, were much greater than the normal means (1.130 and 1.152, respectively).

Of 6 dogs given 3 mg of mercury bichloride for each 100 cc of blood, 5 died. One of those that died had been given only 2 mg of this substance. None of the animals given uranyl nitrate

or potassium dichromate in the doses stated died spontaneously within eight days, although the kidneys of those receiving the larger doses of both poisons showed extensive necrosis in their proximal convoluted tubules.

The activating and inhibiting effects of various substances on renal phosphatase have been studied. Erdtmann<sup>13</sup> discovered that alkaline phosphatase is activated by magnesium in proper concentration, and this has been confirmed by Kay<sup>14</sup> and others. Bamann,<sup>15</sup> Bamann and Heumüller,<sup>16</sup> D. Albers<sup>17</sup> and Massart and Vandendriessche<sup>18</sup> stated that this enzyme is activated by many kations, while Massart and Vandendriessche<sup>18</sup> and Miguel<sup>19</sup> observed that it is inhibited by anions. Thannhauser<sup>20</sup> and his co-workers reported that alkaline phosphatase is activated by iron, manganese, cobalt and nickel ions and is inhibited by zinc and copper. Perlman and Ferry<sup>21</sup> confirmed these observations on manganese and zinc.

Snow and Hepler<sup>22</sup> used multiple dilutions of a solution of 1 milligram-molecule  $\times 10^{-3}$  to study the effects of the poisons used in these experiments on renal phosphatase. They observed that potassium dichromate and mercury bichloride caused definite but relatively slight reduction, while uranyl nitrate in the higher concentration employed induced marked reduction, in the activity of the enzyme. Magnesium chloride used as a control on the same material increased the activity of the enzyme by approximately 50 per cent. In view of these results it is difficult to interpret the consistent increase in the action of phosphatase in the kidneys of dogs poisoned with small doses of these substances. Three possibilities may be considered. Unknown factors in the living animal, not present in the test tube, may influence results. The enzyme may have been protected against the poisons by their combination with proteins of the cells in accordance with the well known phenomenon of precipitation of protein by heavy metals. Or,

13 Erdtmann, H. *Ztschr f physiol Chem* **172** 182, 1927, **177** 211, 1928.

14 Kay, H. D. *J Biol Chem* **93** 733, 1931.

15 Bamann, E. *Naturwissenschaften* **28** 142, 1940.

16 Bamann, E., and Heumüller, E. *Naturwissenschaften* **28** 535, 1940.

17 Albers, D. *Ztschr f physiol Chem* **266** 1, 1940.

18 Massart, L., and Vandendriessche. *Naturwissenschaften* **28** 143, 1940.

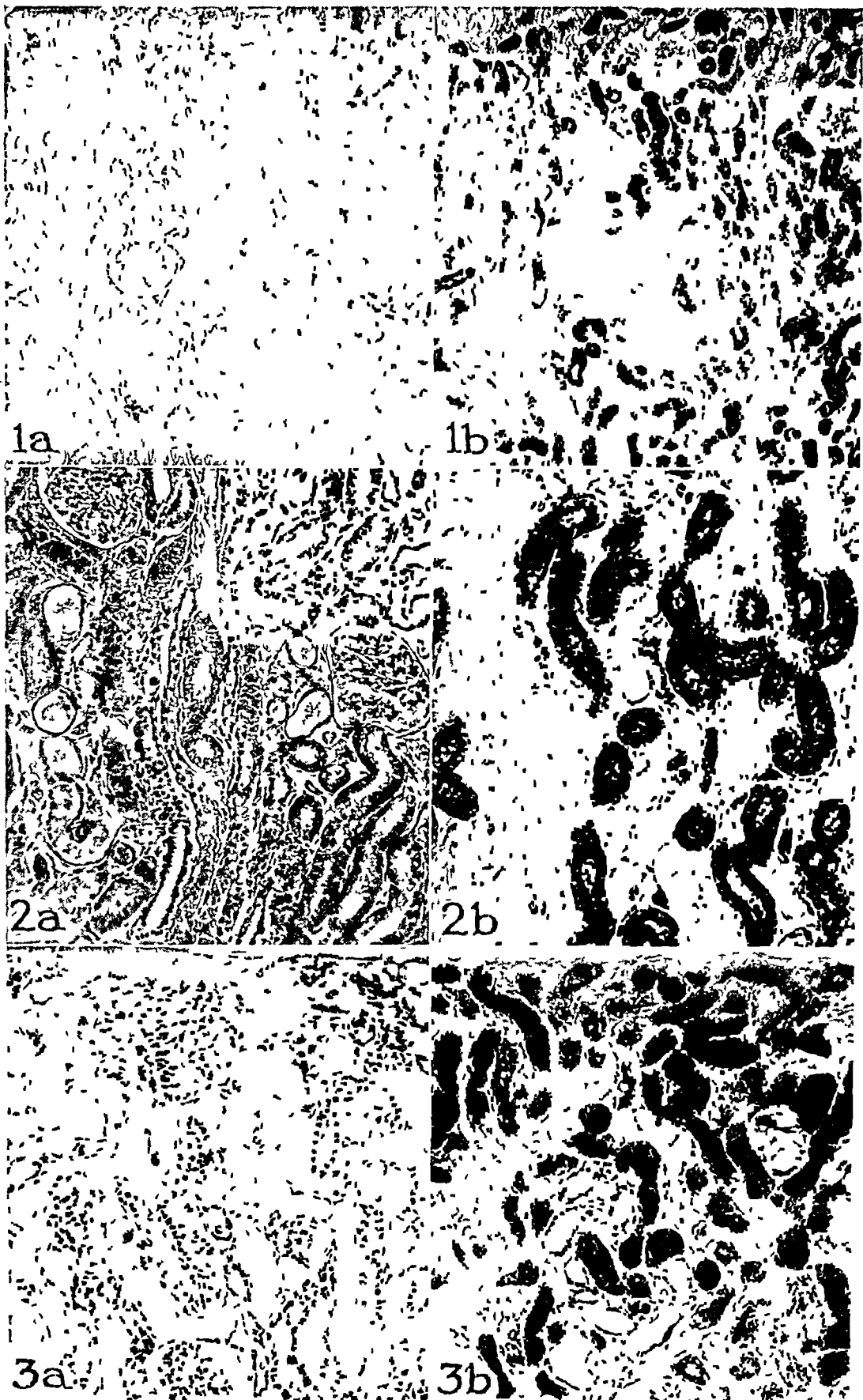
19 Miguel, E. J. *An Asoc quim fram Uruguay* **42** 12, 1940.

20 Thannhauser, S. J., Reichel, M., and Frattin, J. F. *J Biol Chem* **121** 697, 1937.

21 Perlman, G. E., and Ferry, R. M. *J Biol Chem* **142** 513, 1942.

22 Snow, A., and Hepler, O. E. Personal communication to the authors.





Figures 1 to 3  
(See legend on opposite page)

## EXPLANATIONS OF FIGURES 1 TO 3

Fig 1*a*—Section of renal tissue from dog K Hg 6, which received one injection of 3 mg of mercury bichloride to each 100 cc of blood and died on the third day; hematoxylin and eosin stain  $\times 50$ . Nuclei of tubular epithelium within the labyrinth stain well, there is necrosis of the straight terminal portion of the proximal convoluted tubule along the margins of the labyrinth.

Fig 1*b*—Serial section to the one shown in figure 1*a* but stained by Gomori's method  $\times 50$ . The phosphatase in the epithelium of the tubules within the labyrinth is practically normal in amount and distribution, it is markedly reduced in the necrotic epithelium. Chemically, the tissue contains 11.06 units of phosphatase per gram.

Fig 2*a*—Section of renal tissue from dog K Ur 22, which received two injections on successive days, of 3 mg of uranyl nitrate to each 100 cc. of blood and was killed on the fourth day, hematoxylin and eosin stain  $\times 155$ . There is marked necrosis of the straight portions of the proximal convoluted tubules, the nuclei within the labyrinth stain well although the cells are not normal.

Fig 2*b*—Section of renal tissue from the same dog as that shown in figure 2*a*, but stained by Gomori's method  $\times 155$ . There is abundance of active phosphatase with essentially normal intracellular distribution within the labyrinth. Along the margins of the labyrinth the epithelium is necrotic and phosphatase activity is greatly reduced. In the left upper quadrant is a proximal convoluted tubule cut through the beginning of the terminal straight portion. In the upper end of this segment the phosphatase is approximately normal in amount and distribution, in the lower portion the epithelium is necrotic and phosphatase is only very slightly active, between these two portions the phosphatase is diffused through the cytoplasm but is not much reduced in activity. Chemically, the tissue contains 21.70 units of phosphatase per gram.

Fig 3*a*—Section of renal tissue from dog K Cr 16, which received two injections of 3 mg of potassium dichromate to each 100 cc of blood on successive days and was killed on the fourth day, stained with hematoxylin and eosin,  $\times 155$ . There is extensive necrosis of the epithelium of the proximal convoluted tubules in the subcapsular zone and throughout the full length of the tubules.

Fig 3*b*—Section of renal tissue from the same dog as that shown in figure 3*a* but stained by Gomori's method,  $\times 155$ . The phosphatase in the completely necrotic epithelium is active and gives a strong reaction. Chemically, the tissue contains 6.65 units of phosphatase per gram.

finally, the amount of the enzyme may be actually increased by the retention in the necrotic or damaged epithelium of phosphatase which has escaped from the blood in the glomerular filtrate.

If phosphatase in the kidneys has any function it would seem that this must, in some way, be bound up with its distinctive location in the brush border of the epithelium of the proximal convoluted tubules, where it is in immediate contact with the glomerular filtrate in the lumen. The phosphatase of the small intestine is in the superficial cells of the mucosa and is thus strategically located for taking part in the absorption of some substance, possibly dextrose, from the intestinal contents. In other organs, such as the liver (Gomori<sup>2</sup>, Kabat and Furth<sup>23</sup>) and the prostate (Huggins and Johnson<sup>24</sup>, Kutscher and Wolbergs<sup>25</sup>), this enzyme is distributed throughout the cytoplasm of the cells. Freeman, Chen and Ivy<sup>5</sup> found much phosphatase in the bile and believed that the enzyme "originated in the liver." Human semen, particularly the portion from the prostate gland (Huggins and Johnson<sup>24</sup>), contains acid phosphatase in high concentration (Kutscher and Wolbergs<sup>25</sup>, Guttman and Guttman<sup>26</sup>). Thus the acid phosphatase of semen is probably derived from the abundant supply in the cytoplasm of the cells of the prostate. These examples suggest that phosphatase which is uniformly distributed throughout the cytoplasm (liver and prostate) is either a product, or is concerned with the production of a substance, which is discharged into a lumen as an external secretion. On the other hand, the location of phosphatase in the brush border of the cells of the proximal convoluted tubules and in the superficial cells of the intestinal mucosa suggests that this enzyme in these organs is concerned with the absorption of some substance or substances from a lumen. Its alleged relation to absorption of dextrose from the glomerular filtrate and to deposition of calcium in the kidneys will be considered in subsequent papers in this series.

As this paper was being completed, a contribution from Russell, Rouse and Read<sup>27</sup> appeared. After referring to our previous paper<sup>9</sup> in which we stated that phosphatase could still be demonstrated by chemical and histochemical methods in cells rendered necrotic by salts of heavy metals,

these authors continued "This observation suggests an extrarenal origin for the alkaline phosphatase in the kidney since apparently the maintenance of the enzyme is not dependent on viable renal cells." It is not particularly surprising that an enzyme should remain active in dead cells since Moore, Goldstein and Cantowitz<sup>28</sup> have shown that marked histologic changes in the cytoplasm and the nuclei of renal epithelium may occur before the mitochondria—very intimate internal structures of the cell—show any pathologic alteration other than that of position, i.e., diffusion through the cytoplasm. Russell and his co-workers<sup>29</sup> found phosphatase in tubercles but "concluded that alkaline phosphatase does not appear in areas of caseation simultaneously with the development of necrosis of the cells." They stated that "there is some evidence to indicate that alkaline phosphatase in areas of caseation is not of autochthonous origin but is probably derived from the serum, the enzyme diffusing into the area of caseation." However, these authors<sup>29</sup> dismissed the idea of an extrarenal origin of phosphatase with the statement that "it seems most unlikely that the high concentration of alkaline phosphatase in the kidney could be totally explained by a special affinity of certain renal cells to pick up and store the enzyme that had been made in other cells of the body and transported to the kidney by the blood." While we did not make such a suggestion in our previous paper, we had formulated a concept of a possible extrarenal origin of renal phosphatase by an entirely different line of reasoning. The idea is not as fantastic as Russell and his co-workers imply by their comment.

Since foreign proteins with relatively large molecules, e.g., egg albumin, introduced into the blood stream are known to pass through the glomerular filter (Babcock<sup>30</sup> and others), it is likely that the alkaline phosphatase of the plasma may also escape through the glomeruli. It is present in the urine. The amount of a substance which the tubules are capable of absorbing is limited. There is also evidence that there is a limit to the size or the weight of the molecules which they can reabsorb from the glomerular filtrate. The largest molecule which the tubules regularly reabsorb is dextrose with a molecular weight of 180. The maximum capacity for reabsorption (the threshold value) of dextrose is about 180 mg per hundred cubic centimeters,

23 Kabat, E. A., and Furth, J. *Am J Path* **17** 303, 1941.

24 Huggins, C., and Johnson, A. A. *Am J Physiol* **103** 574, 1933.

25 Kutscher, W., and Wolbergs, H. *Ztschr f physiol Chem* **236** 237, 1935.

26 Guttman, A. B., and Guttman, E. N. *Endocrinology* **28** 115, 1941.

27 Russell, W. O., Rouse, E. T., and Read, J. A. *Arch Path* **38** 40, 1944.

28 Moore, R. A., Goldstein, S., and Cantowitz, A. *Arch Path* **8** 29, 1929.

29 Russell, W. O., Read, J. A., and Rouse, E. T. *Arch Path* **38** 31, 1944.

30 Babcock, C. *Anat Rec* **71** 233, 1938.

or 0.001 mol Stieglitz<sup>31</sup> injected a solution containing ferric ammonium citrate, which has a molecular weight of 724, into the veins of rabbits. He stated that this substance was excreted by the tubules. However, his illustration shows iron, rendered visible by the prussian blue reaction, sharply limited to the brush border and the immediately underlying very narrow zone as if it had penetrated the cells from the contents of the lumen. Inulin has a molecular weight of about 5,000 (Westfall and Landis<sup>32</sup>), but because of the great length of its molecule it has a diffusion coefficient equivalent to a molecular weight of about 15,000 (Bunim and co-workers<sup>33</sup>). Although inulin passes through the glomeruli readily, it is not reabsorbed by the tubules. Albers and Albers<sup>34</sup> have stated that the molecular weight of phosphatase is between 6,000 and 10,000.

Since substances of similar and even lower molecular weight are known not to be reabsorbed from the glomerular filtrate by the tubules it is possible that renal epithelium is unable to transfer such a large molecule as phosphatase through the cell substance into the surrounding tissue spaces. Stieglitz<sup>31</sup> illustration strongly suggests the inability of the cells of the proximal convoluted tubules to transport molecules of ferric ammonium citrate which may have diffused through the brush border and cell membrane through the remaining thickness of the cell even with the aid of the osmotic "pull" of the plasma proteins in the peritubular capillaries, augmented as it is by loss of water in the glomeruli. If a suitable method were available, it would be interesting to know whether the large molecules of inulin become entangled in the brush border and thus become concentrated in the same location where phosphatase is normally found. All this suggests the possibility that the presence of phosphatase in the brush border of the epithelium of the proximal convoluted tubules may represent an abortive attempt to reabsorb a substance of high molecular weight which has been excreted by glomerular filtration. If this admittedly bizarre and incongruous idea is correct, the pres-

ence of phosphatase in the kidneys may have no functional significance.

#### SUMMARY

All the experiments reported in this paper were made on dogs and the conclusions apply only to that species.

Changes above and below the normal mean occurred in the alkaline phosphatase of the blood serum within a period of eight days after poisoning with mercury bichloride.

Doses of potassium dichromate and of mercury bichloride that caused extensive necrosis of the epithelium of the renal tubules reduced the phosphatase activity of the kidneys. Uranyl nitrate in doses up to 3 mg to 100 cc of blood caused marked necrosis of the tubular epithelium but did not reduce the phosphatase activity of the kidneys as determined by chemical methods, in many animals the poison appeared to increase it.

Subnecrotizing doses of all three of the aforementioned chemical agents increased the activity of phosphatase in the kidneys, as determined by chemical methods.

The earliest morphologic change in the phosphatase in the epithelium of the proximal convoluted tubules induced by the heavy metal poisons used in these experiments was diffusion of the enzyme throughout the cytoplasm from its normal location in the brush border.

Active phosphatase can still be demonstrated by histochemical methods (Gomori's stain) in the debris of disintegrated necrotic cells of the proximal convoluted tubules.

The function of alkaline renal phosphatase is unknown. If it has a function, it would seem that this must be related to its limitation to the brush border of the epithelium of the proximal convoluted tubules and that it is concerned with absorption of some substance or substances from the contents of the lumen rather than the excretion of products into the lumen.

Reasons are presented for the possibility that alkaline renal phosphatase may not have any functional significance. Its presence in the brush border of the epithelium of the proximal convoluted tubules may be due only to an attempt by these cells to absorb it, the attempt being unsuccessful because its molecule is too large to be transported by the cells from the lumen to the peritubular tissue spaces.

31 Stieglitz, E. J. *Am J Anat* **29** 33, 1921.

32 Westfall, B. B., and Landis, E. M. *J Biol Chem* **116** 727, 1936.

33 Bunim, J. J., Smith, W. W., and Smith, H. W. *J Biol Chem* **118** 667, 1937.

34 Albers, H., and Albers, E. *Ztschr f physiol Chem* **232** 165 and 189, 1935.

# ISCHIOFAGUS TRIPUS

## REPORT OF TWO CASES

CAPTAIN HANS G SCHLUMBERGER

MEDICAL CORPS, ARMY OF THE UNITED STATES

AND

J ELMER GOTWALS, M D

PHOENIXVILLE, PA

An ischiopagus is an unusual type of symmetric diaxial monster in which fusion has taken place in the region of the perineum, with involvement of the bony pelvis. A secondary perineum consisting of tissue derived from both twins is present between the adjacent legs of each side, giving the impression of two pairs of legs and of genitalia arranged at right angles to the main axis of the body. This is the so-called cruciate monster or ischiopagus tetrapus. Actually, only one limb of each pair belongs to either of the individuals. If the main axes of the trunks meet at an angle, the two legs on the side of the smaller angle may fuse into a composite limb, resulting in the form known as ischiopagus tripus. These relations are indicated in the accompanying diagram (fig 1)

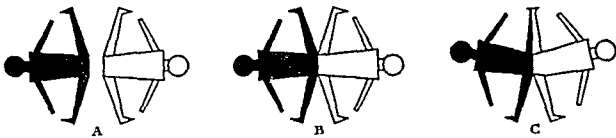


Fig 1—Diagram of the relationship of the components of an ischiopagus. A, two autonomous individuals. B, twins joined to form an ischiopagus tetrapus. C, twins with fusion resulting in an ischiopagus tripus. (The diagram is a modification of that published by H. H. Wilder in the American Journal of Anatomy 3 387, 1904)

The earliest authentic case of this monstrosity was born in 1552 and reported two years later by Rueff.<sup>1</sup> In 1882 Taruffi<sup>2</sup> was able to find records of 14 undoubted cases of ischiopagus tripus, one which he overlooked was reported in this country by Ellis<sup>3</sup> in 1871. Most of these monsters lived only a few hours or days, none for as long as a year. The most recent account of the monstrosity is to be found in the Portuguese literature, it was described by Moitas<sup>4</sup> in

1 Rueff, J. De conceptu et generatione hominis, Tiguri, C. Frobenius, 1554, book 6, p. 47.

2 Taruffi, C. Storia della teratologia, Bologna, reg. tip., 1881-1891, vol. 2, pp. 366-403.

3 Ellis, C. Boston M. & S. J. 8 218, 1871.

4 Moitas, A. Folia anat. univ. conimb. (art. 9) 16 1 1941.

1941. The remarkable capacity of the fetus for functional adaptation in response to extensive developmental anomalies is well illustrated by the following 2 cases.

### CASE 1

The mother was a healthy 24 year old white woman whose only previous pregnancy had resulted in a normal, full term infant. The father was apparently well. Among the mother's siblings was one set of twins, but there were none among the father's. The pregnancy was uneventful, and labor set in at the expected time. Six hours after the onset the head could be seen at the vaginal orifice. After birth of the head there was failure of restitution, and each arm was delivered manually. Further obstruction was then encountered, but by the use of traction and rotation the



Fig 2—Photograph of the twins when 2 days old. The twin described as the smaller or right component is on the reader's left.

three legs and the body of the second twin were delivered.

Immediately after birth the twins were cyanotic and motionless. Within a minute or two the larger one began to cry, the smaller one made the facial movements of crying but emitted no sound. The cyanosis disappeared rapidly except for that of the left half of the chest in the smaller twin, which persisted throughout life. The weight at this time was 11 pounds 12 ounces (5,330 Gm.).

The larger twin appeared normal to the level of the umbilicus. It breathed easily and was never cyanotic until shortly before death. The heart rate was 120 to 180 per minute, the sounds were normal in quality. Bottle-fed, it sucked well and cried lustily, although it often slept while the smaller twin was awake. It moved both arms freely, and when it kicked one leg, the composite third leg was also set in motion.

The smaller twin showed left-sided torticollis, no iris was present in the right eye, and in the left the pupil was irregular. Neither pupil reacted to light. No true respiratory excursions were ever observed, although some motion was transmitted by the diaphragmatic movements of the larger twin. When the latter cried, the former became cyanotic. The heart sounds were always muffled and too rapid to count. The sucking movements of this twin were poorly coordinated, and it took practically none of its formula. This member of the pair occasionally contorted its face as if to cry, but no sound was uttered. In general it was less active than the larger twin, yet it moved both arms freely,



Fig 3—The visceral relations as seen at autopsy. The cecum and the ascending colon pass transversely across the abdominal cavity. The umbilicus is to the left (the reader's right) of this structure. The umbilical vein can be seen passing to the liver of the left twin; the umbilical artery disappears in the pelvis. The left-sided position of the liver in the smaller twin is apparent, as is also the very large pericardial sac which completely fills the thoracic cavity.

and its leg synchronously with the composite one. At times it slept while the other was crying and thrashing about. Urine and feces were expelled at normal intervals from the common urethral and anal orifices.

The twins gradually lost weight. Four days after birth the temperature of both rose to 102 F, on the morning of the next day it reached 103 F. Respiration was rapid and the pulse rate 170 to 180. By evening the temperature was 105 F, the respiratory rate 80, and rales were heard at the bases of both lungs of the larger twin. Death occurred that night.

**Autopsy**—The body weighed 8 pounds (3,629 Gm), the over-all length from crown to crown was 47 cm. Rigor and livor mortis were present. The twin forming the right component of the monster was about a third smaller than that forming the left. A single umbilicus marked the area of fusion of the two trunks, which were apparently normal, as were also the upper extremities and the heads except for the abnormality of the eye in the smaller twin. At the level of the umbilicus and at right angles to the main axis of the body were two well formed legs, between which were normal female genitalia and an anus. Both feet showed talipes varus. In the same relative position on the opposite side was a fusiform structure that apparently had arisen from the union of two legs. The foot was represented by a sharply curved fleshy mass, 2.5 cm long, with a single "toe nail" at its tip. At the base of this composite limb there were no vestiges of genitalia or an anus.

When the abdomen was opened, the distended large intestine was found to form a single loop arranged

transversely to the main axis at the level of the umbilicus. Elsewhere in the larger twin the normal relations of the gastrointestinal tract, the liver, the pancreas and the spleen were preserved. However, in the right-sided member there was complete situs inversus of the corresponding organs, which were also much smaller than normal (fig 3). Both small intestines opened into a common cecum, at the proximal end of which were two appendices lying side by side. The cecum opened into a short large intestine, the lumen of which was double and emptied into a single rectum.

The lungs of the larger twin were pale pink, voluminous and covered the precordium. The heart was entirely normal. In the smaller twin the lungs were hidden by an enormous pericardial sac which completely filled the thoracic cavity and contained 70 cc of clear yellow fluid. The lungs, deep purple, were wedged into the costovertebral angle and appeared to be quite airless. Histologically, the characteristic "crumpled paper bag" outline of the alveoli indicated that the lungs had never expanded—evidence that the pericardial effusion existed prior to birth.

The apex of the greatly enlarged heart was directed toward the right. It weighed 46 Gm as compared with the normal heart of the larger twin, which weighed only 20 Gm. Dissection revealed transposition of the great vessels with an overriding aorta, a defect in the interventricular septum (fig 4) and hypertrophy of the wall of the right ventricle. The aortic cusps were normal. The ascending aorta was a massive vessel which was continued cephalad as three large arteries—a right subclavian artery, a right common carotid artery and an innominate artery which arose to the left of these vessels. The arch of the aorta, directed to the right, was smaller than any one of these three arteries and looked more like a branch of the ascending trunk than a continuation of this vessel.

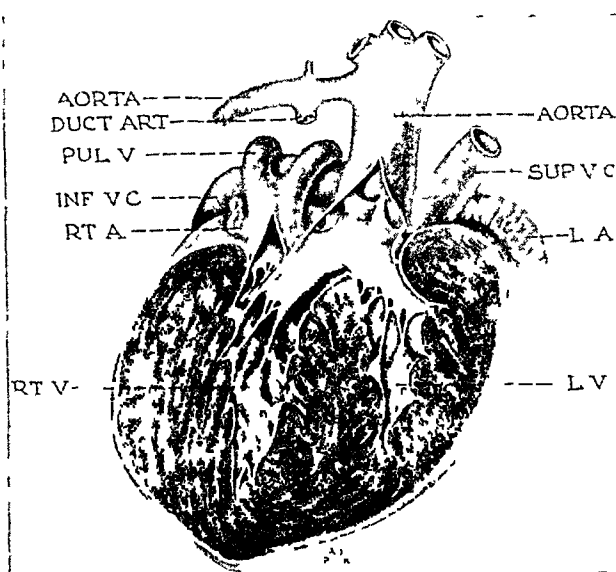


Fig 4—Drawing of the heart of the smaller twin. The section removed the anterior wall of each ventricle. The pulmonary artery which arose from the left ventricle, just anterior to the cut surface, is not shown. *Inf V C*, inferior vena cava; *L A*, left auricular appendage; *L V*, left ventricle; *Pul V*, pulmonary vein; *Rt A*, right auricle; *Rt V*, right ventricle; *Sup V C*, superior vena cava; *Duct Art*, ductus arteriosus.

The pulmonary artery arose from a stenotic opening in the left ventricle guarded by a malformed valve. It communicated with the aorta by a widely patent ductus arteriosus. The pulmonary veins combined to form a single large vein which emptied directly into

the right ventricle. The right auricle was represented by an inconspicuous and irregular little nodule attached to the wall of this vessel. The inferior vena cava joined the superior vena cava just before the latter emptied into the left ventricle. This junction was all that remained of the left auricle, although a fairly well developed auricular appendage was present. At the opening into the left ventricle was a well formed tricuspid valve. At the point of union between the superior and inferior venae cavae there was a valve leaflet so arranged that the blood flowing down the superior vena cava would pass into the inferior vena cava as well as into the left ventricle.

In the region of the pelvis the aorta of the smaller twin was directly continuous with the aorta of the larger by fusion with the right common iliac artery of the latter. It gave off a left common iliac artery which fused with the distal portion of the right common iliac artery of the larger twin to supply the single composite leg. From the left common iliac artery of the larger twin arose the single umbilical artery. Only a single, partly fibrosed umbilical vein was present, forming the ligamentum teres of the larger twin (fig 3). Cross section of the cord likewise showed only one vein and one artery. From this it is apparent that in intrauterine life the placental circulation was directly associated only with the larger twin.

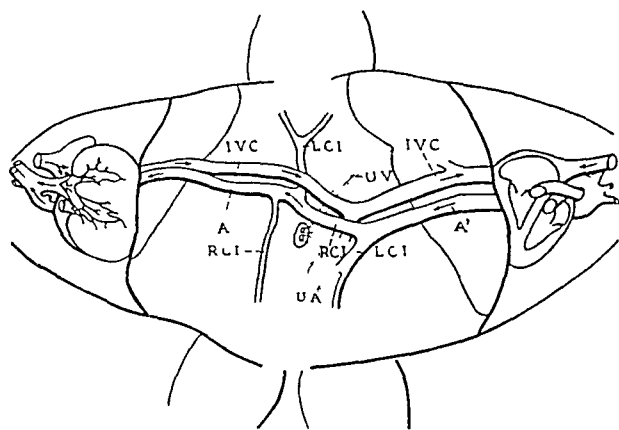


Fig 5—Diagram of the circulation, arrows show the direction of blood flow. The letters prime indicate the vessels in the left member of the monster. *A*, aorta, *I V C*, inferior vena cava, *L C I*, left common iliac artery, *R C I*, right common iliac artery, *U A*, umbilical artery, *U V*, umbilical vein.

The union of the inferior venae cavae was similar to that of the aortas. Before the autopsy was begun, a 35 per cent solution of diodrast was injected into the right brachial artery of the larger twin and a roentgenogram taken. All of the main vessels of both twins could be clearly recognized, as illustrated in figure 5, a tracing made over the roentgenogram.

The transposed liver of the smaller twin weighed 40 Gm, that of the larger, 120 Gm. The gallbladders and biliary passages were apparently normal, as was also the histologic structure of the parenchyma of the livers. The spleens, which weighed 3 Gm and 6 Gm respectively, were grossly and microscopically normal except for the transposition of the smaller one.

A double kidney formed by end to end union was present in the pelvis at the level of the composite limb, its long axis parallel to that of the main axis of the two trunks (fig 6). On section of the kidney the usual corticomedullary relations were present. However, scattered through the parenchyma were numerous discrete white areas (0.5 to 1 mm) which on histologic examination were found to be abscesses. An infiltrate of neutrophils was likewise found in the pelvic mucosa.

The half of the double kidney belonging to the smaller twin possessed two ureters that fused to form a greatly dilated single ureter which opened into the bladder posteriorly. This saclike ureter may well have acted as an accessory bladder. No stenosis of the orifices was present. The remaining half of the kidney had a single ureter which passed behind the sigmoid flexure to open into the bladder anteriorly. Its distal portion for a length of 0.7 cm was greatly constricted yet the lumen was patent. Two urethral orifices were present at the base of the bladder. The urethras soon fused to form a single duct which opened externally in normal relation to the vagina and the rectum.

At each pole of the kidney was a roughly triangular adrenal gland. Two other adrenal glands, circular in outline, were found in their normal positions on the opposite side, their shape was due to the absence of the kidneys on that side.

Two uteri as well as four fallopian tubes and four ovaries were present in the pelvis (fig 6). The tubes

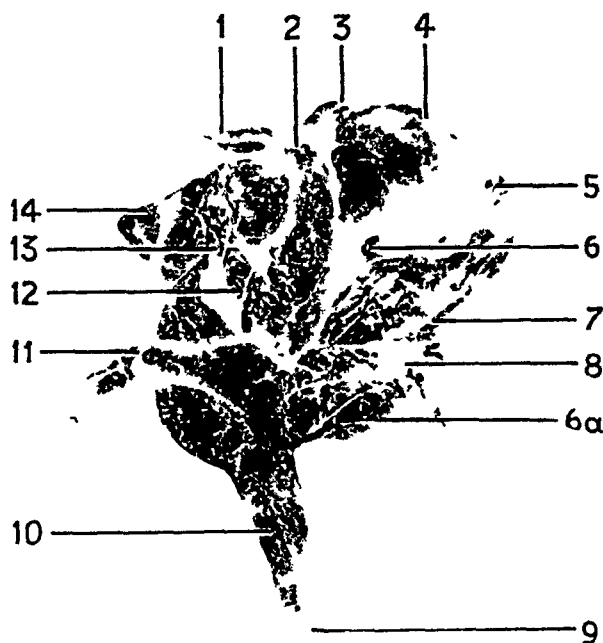


Fig 6—Photograph of pelvic viscera in situ, the adjacent structures have been blocked out. 1, 2, 3, one set of fallopian tubes, ovaries and uterus, 4, kidney, 5, 14, adrenal glands, 6, 12, 13, ureters, 7, rectum, 8, 11, second set of fallopian tubes, ovaries and bicornate uterus, 9, urachus, 10, bladder.

and ovaries were grossly and microscopically normal. The uterus on the side of the composite limb, though derived in part from each of the twins, was well formed and placed at right angles to the main axis of the body. The vagina lay between one of the ureters and the rectosigmoid, it had no orifice and was greatly distended with inspissated mucus. Fusion was incomplete in the second uterus, producing an extreme bicornuate structure in which each half lay parallel to the long axis of the trunk. The twin cervical canals opened into a single vagina, the orifice of which was normally placed between that of the urethra and the anus.

Except for a deformity of the right clavicle of the larger twin the bones of the trunks, the arms and the two legs were normal. The two lumbar vertebrae of each trunk were acutely flexed and laterally rotated. The central epiphyses of the single sacrum were found on the side of the two legs and at right angles to the



rest of the vertebral column. The lateral sacral epiphyses formed an arc that extended from the last lumbar vertebrae to a position lateral to the central epiphyses. The iliac, ischial and pubic bones were normally situated in relation to the sacrum (fig 7).

On the side of the composite limb there was an irregular flat bone, apparently an ilium. The single femur had a poorly developed head and a shaft that flared out broadly at its distal end, where two epiphyses could be recognized. This is the chief evidence that the composite limb actually resulted from the fusion of two leg anlagen early in embryonic development. The proximal end of the tibia was broad, the distal end, narrow. No fibula was present. A single bone could be recognized in the portion of the limb which represented the foot.

The brain of the smaller twin weighed 179 Gm, that of the larger, 375 Gm. In both brains the gross struc-



Fig 7—Roentgenogram showing the bones of the trunk and the composite limb

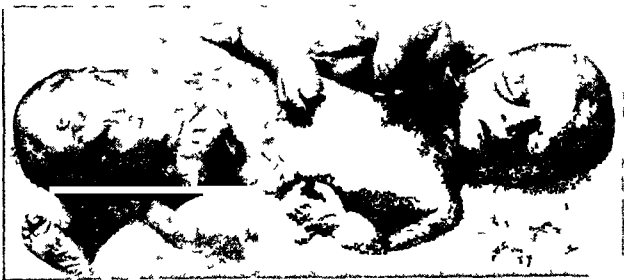


Fig 8—Gross appearance of the ischiopagus tripus in case 2. For a description see the text.

tures and convolutional markings were normal, as were also the circles of Willis. In the brain of the smaller twin the veins were not collected into three vessels, but instead emptied directly into the longitudinal sinus. They were all greatly engorged and were present in such large numbers as to give the appearance of a veritable brush. On histologic examination of the brain substance the striking abnormality was a tremendous dilation of the capillary venous bed. Although these vessels were normal in arrangement, their size and their degree of engorgement gave them a prominence which at first led to the belief that there was also an increase in number. This, however, was more apparent than real.

#### CASE 2

In the teratologic collection of the Army Medical Museum there is another example of ischiopagus tripus (fig 8). Born of Filipino parents these twins survived for forty-six hours after birth, no further details of

their history are available. The teras, which is a female, measured 36 cm from crown to crown. When placed in a position similar to that employed in describing the previous specimen, the twin on the right was again distinctly smaller than that on the left. Likewise, it displayed developmental anomalies absent in the larger twin. There were a cleft palate and lip and a deformed right hand wherein only the thumb was separate, the fingers being united to form a mitten-like structure. The composite limb had two partly fused but recognizable feet, each foot bearing five toes.

On dissection the twin on the right exhibited complete situs inversus, which included both the heart and the abdominal viscera. The heart had a single ventricle and auricle, the lungs were completely collapsed. The aorta was broadly continuous with the right common iliac artery of the larger twin. The venae cavae were likewise confluent. The single umbilical artery arose from the left common iliac artery of this twin, which also received the only umbilical vein that could be recognized. Hence, it is apparent that the circulation was identical with that of the foregoing teras. All abdominal viscera were much smaller in the twin on the right than in that on the left. Fusion of the small intestines took place 6 cm proximal to the cecum. The appendix, the large intestine and the rectum were single. A horseshoe kidney was found in the smaller twin, two normally placed kidneys were present in the larger. An adrenal gland surmounted the upper pole of each kidney, including the two components of the horseshoe kidney. The ureters opened into a single bladder that was transverse to the main axis of the trunks. A bicornate uterus with attached fallopian tubes and two ovaries was in normal relation to the bladder. No trace of a second uterus or adnexa could be found.

#### COMMENT

The ischiopagus belongs to the large group of terata known as symmetric diaxial conjoined twins. These consist of two fairly equally developed individuals that are partly fused. This union may occur in the region of the head (cephalopagus), the thorax (thoracopagus), the umbilicus (omphalopagus), the buttock (pygopagus) or the perineum (ischiopagus). Numerous subdivisions and interstages of these forms have been described. Both members are always of the same sex, which favors the view that they are derived from a single ovum, as are homozygous (identical) twins. This assumption also gains support from the fact that junction is always effected by a union of like parts, e g, head to head, never head to buttock. The absence of haphazard fusion indicates that in every instance the developing embryos must have had parallel axes. This can be accounted for only by assuming a duplication of the developing ovum by fission some time between the two cell stage and the formation of the primitive streak. The subsequent union of the two individuals probably occurs during the first three weeks of development.

Considerable experimental evidence has been accumulated in support of these theories. In 1915 Huber<sup>5</sup> opened the uteri of rats at varying



intervals after copulation. In 1 instance he found the two blastomeres resulting from the first cleavage distinctly separated by a space equal to about half the diameter of each of the cells. They were normal in size, shape and structure, as also were their nuclei. The two cells lay free in a slightly distended portion of the lumen and did not appear to have been separated as a consequence of manipulation. The authors suggested that each might have developed into a complete fetus, with production of identical twins. This possibility has since been realized by the work of Nicholas and Hall.<sup>6</sup> These investigators removed ova that were in the two cell stage from rat uteri and placed them in a slightly acid solution of sodium and potassium chlorides. The zona pellucida dissolved off in about three hours, and the two blastomeres could be readily separated by a jet of water. When returned to the uterus each underwent normal development. These authors also demonstrated the possibility of further development after fusion of two ova. Since they used unsegmented eggs rather than young embryos, they obtained a single giant fetus rather than a conjoined monster.

The production of double monsters by fusion of embryos in the gastrula stage was first carried out by Spemann in 1918, using the eggs of the salamander Triton. This material was reexamined and supplemented by Wessel,<sup>7</sup> who showed that when the blastopores of the two ova were placed in varying relations to each other, the type of double monster that would result from each relationship could be accurately predicted. The well known two-headed salamander first produced by Spemann in 1903 by constructing the egg with a hair loop is not relevant to this discussion since it is the result of a partial duplication of a single axis rather than of the fusion of two.

In the cases reported here there is complete situs inversus of the right component. As long ago as 1865 Forster<sup>8</sup> emphasized the frequency of situs inversus in one member of a double monster and stated that this occurred only in the twin on the right. Although there are exceptions to the rule limiting situs inversus to the individual on the right of a double monster, it is true for about 90 per cent of the cases. This interesting localization of the visceral anomaly was lost sight of by investigators until 1919, when Morrill<sup>9</sup> described it in three cruciate monsters of

the fish *Fundulus* and a human dicephalus tribrachius. In the same year appeared a paper by Spemann and Falkenberg<sup>10</sup> on situs inversus in twins and double monsters. The authors provided a satisfactory explanation of this phenomenon based on their findings in identical twins and monsters experimentally produced in the salamander. The median limbs and trunk muscles of twins resulting from the division of a Triton embryo are poorly developed, whether this division occurs in the late gastrula or even in the two blastomere stage. Hence the twins, though separate, can be recognized as right and left. Of 25 "left" twins, 1 showed inversion of the heart, of 30 "right" twins, 14 had complete situs inversus. Of 12 double monsters with anterior duplication, the left portion was normal in all and the right showed situs inversus in 10. The impaired development of the trunk musculature of the adjacent sides of the two twins resulted in a bending of the trunk away from the midline. In the left twin this was toward the left, in the right it was directed to the right. In both, subsequent development restored the normal position of the trunk. The first evidence of normal asymmetry is found in a twisting of the liver anlage to the right associated with a bending of the gut to the left. In the case of the left component of twins or double monsters the abnormal curvature of the trunk to the left merely accentuates this normal asymmetry. However, in the right component the normal displacement of the gut to the left is counterbalanced by the bending of the trunk as a whole to the right. If this is sufficiently marked, the gut will be pulled to the right, and the liver anlage will take up a position to the left of it, leading to situs inversus viscerum.

This explanation of situs inversus does not apply to the same condition when found in an autonomous individual. In view of the prevalence of the anomaly in conjoined twins it was at one time suggested that when the anomaly was found in an otherwise normal person one could assume that the person was one of twins the other having died early in development and been resorbed. This theory has been discredited and Cockayne<sup>11</sup> has brought forth evidence to show that in these cases the tendency is inherited as a recessive characteristic.

Both the specimens of ischiopagus tripus here reported were female. Of the 14 recorded by Taruffi,<sup>2</sup> 11 were female, the sex of the remaining 3 is unknown. The one recently described by Moitas<sup>4</sup> may have been male. This preponderance of the female sex is found in most types of double monsters. It can probably be explained

6 Nicholas, J. S., and Hall, B. V. *J. Exper. Zool.* **90** 441, 1942.

7 Wessel, E. *Arch. f. Entwickl. mechn. d. Organ.* **107** 481, 1926.

8 Forster, A. *Die Missbildungen des Menschen systematisch dargestellt*, ed 2, Jena, F. Mauke, 1865, p. 136.

9 Morrill, C. V. *Anat. Rec.* **16** 265, 1919.

10 Spemann, H., and Falkenberg, H. *Arch. f. Entwickl. mechn. d. Organ.* **45** 371, 1919.

11 Cockayne, E. A. *Quart. J. Med.* **7** 479, 1938.

in part by the fact that male fetuses are less likely to survive in the presence of developmental anomalies than are females. Potter and Adair<sup>12</sup> stated that stillborn fetuses delivered early in pregnancy are much more commonly male than female. Under four months 78 per cent are male. Many of the male monstrosities are therefore aborted at such an early date that anomalies are not readily recognized and are discarded by the physician. Furthermore, in the cases of miscarriage of early pregnancy the physician frequently does not even see the fetus, as he is called in only when persistent bleeding follows incomplete abortion.

The circulatory systems of these terata were remarkable for the broad union of the aortas via the right common iliac artery of the larger twin. Of added interest is the fact that in the case reported by Moitas a precisely similar union had occurred, indicating that given a certain set of conditions the same result will follow, whether in normal or in abnormal development. In view of the complete atelectasis of the lungs in the smaller twin, it was only the union of the aortas that made survival of the monster possible. Without it the intrauterine development would likewise soon have ended since the single umbilical vein and artery were in direct communication only with the blood supply of the larger twin. Although the direction of the flow of blood in the aorta and the inferior vena cava of the smaller twin was reversed, the blood in the aorta was arterial and that in the vena cava venous. If it is true that the blood flowed toward the heart in the descending aorta, then all its blood as well as that from the ascending aorta must have passed to the arms and the head. That this actually took place is indicated by the large caliber and engorgement of the cerebral vessels of the smaller twin in one of the monsters.

The heart of the larger twin was normal, that of the smaller, distinctly abnormal. Besides the transposition of the great vessels in the first case there were overriding of the aorta, a defect of the interventricular septum, stenosis of the pulmonary orifice and hypertrophy of the wall of the right ventricle. These findings can be classified together as forming the tetralogy of Fallot. Cockayne<sup>11</sup> pointed out that the most common cardiac anomaly in cases of complete situs inversus in an autonomous individual is, in fact, the tetralogy of Fallot. The author stated further that cardiac anomalies are present in almost 10 per cent of cases showing transposition of the viscera.

The great pericardial effusion surrounding the anomalous heart of the first monster was not associated with any inflammatory reaction. However, in the epicardium were very many greatly distended thin-walled vascular channels. Comparison with the same region in the normal heart of the larger twin showed that these vessels were five times as numerous in the abnormal heart and their calibers many times greater. The dilation of these veins and the increased permeability of their walls due to the anoxemia of this twin before and after birth may account for the large transudate present in the pericardial sac.

The presence of a fused lateral kidney in the first case and of a median horseshoe kidney in the second fits in well with the modern concept of the formation of compound kidneys as described by Lewis and Papez<sup>13</sup>. These investigators, basing their interpretation on the study of a large series of pig embryos, stated that the renal anomaly was due to the relation of the kidneys to the aorta where the latter divides into the common iliac arteries. This bifurcation forms a crotch in which the kidneys are lodged and from which they escape by migrating upward. The arteries, therefore, act as a mechanical obstruction which tends to bring the renal blastemas close together, so that fusion may readily take place. A precisely similar condition was present in the first case here described. The right renal blastema of one individual and the left of the other were caught in the crotch of the partly fused common iliac arteries that supplied the composite limb. Hence a lateral union resulted after the manner of the more common median fusion in autonomous individuals as is seen in one member of the second ischiopagus here reported.

The incomplete double ureter of one component of the laterally fused kidney in case 1 may be accounted for by an exaggeration of the normal bifurcation of the tip of the ureteral stalk. Instead of being confined to the primitive pelvis the fission was continued into the ureter. We can offer no explanation for the unilateral renal agenesis.

#### SUMMARY

In 2 cases of ischiopagus tripus the right member of the pair suffered a complete situs inversus viscerum associated with extensive cardiac anomalies. Likewise, in each teras there was an end to end fusion of the aortas so that the flow of blood in the aorta of the member on the right was reversed.

<sup>12</sup> Potter, E. L., and Adair, F. L. *Fetal and Neonatal Death*, Chicago, University of Chicago Press, 1940, p. 4.

<sup>13</sup> Lewis, F. T., and Papez, J. W. *Anat. Rec.* 9: 105, 1915.

# STUDIES ON THE PATHOGENESIS OF GLOMERULONEPHRITIS

## I PRODUCTION OF AUTOANTIBODIES TO KIDNEY IN EXPERIMENTAL ANIMALS

PHILIP A. CAVELTI, M.D., AND ELSE STAEHELIN CAVELTI

SAN FRANCISCO

In reviewing the literature which deals with the numerous attempts of many investigators along several distinct lines of experimentation to produce glomerulonephritis in animals, it becomes apparent that only those procedures in which specific antibodies to kidney were employed as the pathogenic agent have met with any notable and consistent success. Although renal lesions have been produced by the use of various micro-organisms and nontoxic antigens, such as foreign serum, these changes have not been shown to be progressive as is human glomerulonephritis in a considerable proportion of cases. However, the lesions obtained by means of antikidney serums have been shown<sup>1</sup> to be of a severe and frequently progressive nature and are regarded as resembling human glomerulonephritis in many respects much more than the changes produced by agents other than renal antibodies.

The possibility therefore suggested itself that human glomerulonephritis might be due to antibodies reacting specifically to renal material. Such an interpretation, however, seemed difficult, particularly as to the formation of such antibodies, since in the experiment the antikidney serum had to be prepared in an animal species other than the one in which the nephritis was to be produced.

If human glomerulonephritis is caused by specific antikidney antibodies, it would have to be assumed that the subject's own kidneys furnish the antigen for the production of these anti-

bodies. Investigations have been undertaken wherein an attempt was made to produce antibodies in response to kidney of the same species by means of repeated injections of rabbit kidney, either fresh or autolyzed,<sup>2</sup> in rabbits, however, the results were entirely negative both with respect to the formation of renal antibodies reacting *in vitro* and with respect to the production of nephritis.

The first evidence in favor of the possibility of antigenic action of renal material of the same species under certain circumstances was brought forth by Schwentker and Comploier.<sup>2b</sup> They repeatedly injected mixtures of rabbit kidney emulsion and staphylococcus toxin into rabbits. By means of complement fixation, with plain rabbit kidney emulsion used as an antigen, they recorded positive reactions with the serum of the immunized animals up to a maximal dilution of serum of 1:80. But when the treatment consisted of injections of mixtures of rabbit kidney and streptococcus toxin (Dick toxin) they obtained only very faint reactions up to a serum dilution of 1:10. No reaction was detected with serum from rabbits which had been treated with either one of the toxins alone or with rabbit kidney alone.

The purpose in the present studies has been (1) to investigate more closely the possibility of a production of autoantibodies to kidney incited by homologous kidney rendered antigenic by combination with foreign antigens—particularly streptococci and their products—and (2) to ascertain whether any antibodies to kidney formed have the ability to react with the kidney *in vivo*, thus precipitating nephritis.

The studies have been carried out simultaneously in two animal species, rabbits and rats, by means of essentially analogous procedures.

### MATERIALS AND METHODS

*Preparation of Renal Material*—In order to prevent any extensive interference by the antigens of the blood, the kidneys which were to be used for immunization

<sup>1</sup> Wilson, G. W., and Oliver, J. J. *Exper Med* **32** 183, 1920. Masugi, M. *Beitr z path Anat u z allg Path* **91** 82, 1933, **92** 429, 1934. Smadel, J. E. *J Exper Med* **64** 921, 1936, **65** 541, 1937. Swift, H. F., and Smadel, J. E. *ibid* **65** 557, 1937. Smadel, J. E., and Farr, L. E. *ibid* **65** 527, 1937. Smadel, J. E., and Swift, H. F. *ibid* **74** 345, 1941. Ehrlich, W. E., Wolf, R. E., and Bartol, G. M. *ibid* **67** 769, 1938. Hemprich, R. *Ztschr f d ges exper Med* **95** 304, 1935.

<sup>2</sup> (a) Parks, A. E., Ethridge, C. B., and Taussig, B. L. *Proc Soc Exper Biol & Med* **35** 418, 1936. (b) Schwentker, F. F., and Comploier, F. C. *J Exper Med* **70** 223, 1939.

and serologic work were perfused until they were free of blood. This was done while the animal was under ether anesthesia by inserting a cannula either into the aorta near the branching of the renal arteries or, more recently, into the left ventricle. An outlet was provided by opening the right auricle. Isotonic solution of sodium chloride at 38 to 40 C and under pressure of about 120 cm of saline solution was used. If the perfusion was begun while the animal was still alive, the kidneys always became very pale in a few seconds. Perfusion was continued for at least three to five minutes.

The kidneys were stripped of their capsules, weighed and then ground in a mortar with sand. Saline solution was added gradually to make a 20 per cent suspension by weight. Coarse particles were eliminated by short centrifugation. The whole procedure was carried out under strictly sterile conditions. Sterility tests of the emulsions were set up. The materials then were immediately frozen and kept at  $-76^{\circ}\text{C}$  in the carbon dioxide ice box.

*Preparation of Streptococcic and Other Bacterial Antigens*—In order to prevent the introduction of antigens other than those of streptococcic origin, the organisms (group A beta hemolytic streptococci) were grown on a synthetic medium prepared according to the description by Bernheimer<sup>3</sup>. The medium consisted in the main of casein hydrolysate, additional amino acids (glutamine was found to be essential), salts, vitamins and dextrose. The acids which formed during the growth of the organisms were periodically neutralized. A heavy growth was obtained by this method. The organisms either were centrifuged out and resuspended in a smaller volume of saline solution or were concentrated in the medium by centrifugation. The supernatant medium was passed through a Seitz filter and used as a toxin. The bacterial suspensions that were used were from 2 to 7 per cent packed bacteria when centrifuged in Hopkins tubes. The bacterial suspensions usually were kept frozen at  $-76^{\circ}\text{C}$ .

*Staphylococcus* strain "Wood" was grown on Bernheimer's synthetic medium for streptococci modified slightly to contain less dextrose (0.002 per cent) and in addition, 0.08 per cent aminoacetic acid. The filtrate of these cultures in amounts of 0.3 cc per kilogram of body weight killed rabbits in two hours.

*Serologic Tests for Antibodies to Kidney*—The collodion method was used predominantly for the detection and the determination of antibodies to kidney. Other serologic tests occasionally were employed for confirmatory purposes. The collodion particle technique as used in these studies, especially with regard to the preparation of suitable particles (giving no nonspecific reaction), as well as to the optimal proportions of the components of the admixture, has been described in detail in another paper<sup>4</sup>. A brief outline of the test follows. As an antigen for the determination of antibodies to kidney, extracts of rabbit or rat kidney were used which had been prepared simply by centrifugation of the kidney emulsion until the supernatant was clear. When this was difficult to achieve, the supernatant was filtered through paper or a Seitz filter. These extracts (of 20 per cent kidney emulsions) had a protein content of approximately 0.2 per cent. Equal amounts of collodion stock suspension (of a density to match

McFarland<sup>5</sup> scale no. 2 when diluted 1:20) and kidney extract were mixed immediately before use. The mixture was diluted 1:10 or 1:20 and added in amounts of 0.2 cc to 0.5 cc of the diluted serum, the latter having been prepared by the doubling dilution method in agglutination tubes, usually beginning with a dilution of 1:10. The tubes were shaken, incubated for one hour at room temperature and then centrifuged for three minutes at 1,400 revolutions per minute. The agglutinations were read while the sedimented particles were resuspended carefully by shaking.

*Controls*—Each serum was tested in low dilutions (1:10 to 1:80) for nonspecific reactions with plain collodion particles. As soon as significant reactions of this sort were noted, new collodion particles were prepared. In addition, with each label of serum one or two normal serums were tested similarly with collodion antigen. Controls comprising extract of kidney plus collodion particles in saline solution also were set up. The titers were recorded in terms of serum dilutions with disregard for the further dilution which took place on the admixture of the antigen.

Satisfactory performance of the collodion method with tissue antigens had been investigated first with rat kidney and rabbit anti-rat kidney serum.

#### EXPERIMENTAL DATA

A total of 83 rabbits in groups comprising 3 to 10 animals were immunized with the following materials:

1 A strain of beta hemolytic streptococci isolated from a patient with sore throat, killed by heat (two hours at  $60^{\circ}\text{C}$ ) and mixed with rabbit kidney emulsion.

2 Ten separately grown strains of group A, beta hemolytic streptococci isolated from various persons with streptococcic infections, mixed, killed by heat and admixed with rabbit kidney emulsion.

3 The last-mentioned strains, mixed, killed with 1 per cent by volume of chloroform, admixed with rabbit kidney emulsion and incubated overnight at  $37^{\circ}\text{C}$ .

4 Strain NY 5 of beta hemolytic streptococci, killed with 10 per cent by volume of ether and mixed with rabbit kidney.

5 *Streptococcus* strain NY 5, living, mixed with kidney.

6 Dick toxin (filtrate of culture of strain NY 5) plus rabbit kidney.

7 *Streptococcus* strain NY 5, killed by ether and mixed with "kidney broth." The broth was prepared by heating the kidney emulsion in the autoclave at 20 pounds (9 Kg) of pressure for three hours. The precipitate was removed entirely by passage through a Seitz filter. The filtrate was practically free of protein.

8 *Streptococcus* strain NY 5 grown on rabbit kidney emulsion for twenty-four hours, with and without admixture of Bernheimer's synthetic medium to enhance growth, and killed by addition of ether. In some instances, the material was precipitated with alum instead of the admixture of ether.

9 Three other strains of group A streptococci grown on rabbit kidney emulsion and killed by ether.

10 A Seitz filtrate of rabbit kidney emulsion on which strain NY 5 had been grown.

11 *Staphylococcus* toxin mixed with rabbit kidney.

12 Hog serum mixed with rabbit kidney.

13 Rabbit kidney emulsion.

3 Bernheimer, A. W., Gillman, W., Hottle, G. A., and Pappenheimer, A. M., Jr. *J. Bact.* **43**: 495, 1942.

4 Cavelti, P. A. *J. Immunol.* **49**: 365, 1944.

5 McFarland, J. *J. A. M. A.* **49**: 1178, 1907.

14 The same strains of beta hemolytic streptococci as used in combination with kidney alone, killed by heat, killed by ether, and living

15 Staphylococcus toxin

*Proportions of the Components of the Admixtures*—Various proportions were used. The foreign antigen was admixed in amounts of about one tenth to one sixth of the amounts of renal material in terms of dry residue of each. After admixture the preparations, if not used immediately, were kept frozen at  $-76^{\circ}\text{C}$ .

*Schedules of Immunization*—In the earlier experiments injections were given twice weekly. With many rabbits these injections were continued until the animals had received more than forty injections. Schedules comprising three injections each week on successive days, a rest of one week being allowed after every two weeks, and schedules of five to ten injections on successive days, also were employed. Sometimes this treatment was repeated after a rest period of two to six weeks or more.

*Mode of Injection and Dosage*—The injections were given intra-abdominally, 0.3 to 10 cc of the antigen preparations (mixtures) were given each time, the maximal dose in most cases not exceeding 6 cc.

*Bleeding*—The animals were bled from the ear vein on the fifth to eighth day after the last injection. In some cases frequent bleedings were carried out to determine the peak of antibody production.

*Control Tests*—Before immunization the serum of each rabbit was tested for the presence of antibodies to kidney. In no case of untreated rabbits could any such antibodies be detected.

RESULTS OF IMMUNIZATIONS OF RABBITS

The serum of each rabbit was tested serologically after every two to six injections. None of the 17 control rabbits treated with streptococci, staphylococcus toxin or plain rabbit kidney showed any evidence of production of antibodies to kidney although some of them had been immunized for a long time and with considerable amounts of the antigen (up to fifty injections).

In all rabbits immunized with mixtures of a streptococcic antigen and rabbit kidney, antibodies to kidney developed, which were detectable by means of the collodion agglutination technic, an extract of plain normal rabbit kidney being used as an antigen for the test. The number of injections which were necessary varied considerably, but in several cases such antibodies were detected after two injections had been given, four days apart. No tests have been performed as yet after one injection. The maximum titer was reached after six to twenty injections, however, most of the animals reached their highest titer after twelve to eighteen injections. Subsequent, prolonged immunization usually resulted in a reduction of the titer, but with some exceptions. The height of the maximum titers varied from 1:80 to 1:40,960 in terms of serum dilutions. In a great majority of the cases it lay between 1:160 and 1:1,280.

As determined serologically, preparations containing whole streptococci killed by heat, ether or chloroform and mixed with kidney showed the highest activity in producing antibodies to kidney. The immunization with living streptococci mixed with kidney yielded lower titers. Considerably lower titers were also obtained with Dick toxin plus kidney and staphylococcus toxin plus kidney. Streptococci plus kidney broth produced good titers. Treatment with the preparations consisting of streptococci grown on rabbit kidney resulted in most instances in low titers. Hog serum plus kidney gave only doubtful results. (Table 1 gives a summary of some of the maximal titers observed in rabbits.)

TABLE 1—*Titers of Renal Antibodies of Some Rabbit Serums in Terms of the Highest Dilution of Serum or Antigen Yielding a Positive Result*

Rabbit	Immunized with	Collo- dion Technic	Highest Dilution of Serum in Which Aggluti- nation of Kidney Cells Occurred	Precipitin Test (Highest Dilution of Antigen Yielding Precipi- tation)
13	Rabbit kidney + killed streptococci	1:280		80
12		640		
14		1,280	640	40
16		320		
20		40,960	1:280	160
43		160	80	
38		20	160	
45	Dick toxin + rabbit kidney	1,280	160	
68		320	160	
47		40	160	
28		160		
33	Rabbit kidney broth + killed streptococci	160		
36		1:280		
39		320		
2	Staphylo toxin + rabbit kidney	80		
24		320		
50		80		
76	Hog serum + rabbit kidney	20		
78		20		
9	Rabbit kidney	0	0	0
10		0		
17	Streptococci	0	0	0
26		10 (?)		

OTHER SEROLOGIC TESTS FOR ANTIBODIES TO KIDNEY

A number of rabbit serums were tested for agglutination of suspensions of rabbit kidney cells.

Perfused rabbit kidney, which had been kept frozen, was ground and suspended in saline solution. The particles were washed two to three times by centrifugation. Coarse particles were eliminated by centrifugation for one minute at 1,500 revolutions per minute. When tested shortly after preparation, such suspensions, which consisted mostly of fragments of kidney cells,

gave good agglutinative results. The suspension was admixed in appropriate density to the serum dilutions. After incubation of about one hour at room temperature the agglutination tubes were centrifuged at 1 400 revolutions per minute for three minutes, and the agglutinations were read while the tubes were shaken.

In essence the results paralleled those found with the collodion method. Some differences in titer were observed, however, also, more tendency toward nonspecific agglutination was noted.

In a few cases, antigen dilution precipitin tests were carried out with extract of kidney as an antigen. With serums that were relatively high titered in terms of collodion agglutination precipitation was obtained, it was, however, usually of a low order, occurring with antigen dilutions not exceeding 1 40 to 1 160. As these dilutions refer to the extract prepared by centrifugation

A limited number of serums have been tested for species specificity with extracts of rat kidney. No reaction has been noted.

Approximately 40 serums have been compared by means of the collodion method with respect to their content of antibodies to kidney and antibodies to the streptococcus used for immunization. No consistent parallelism was found between the two types of antibodies, some serums of high titer of antibodies to kidney showed low titers of streptococcic antibodies, and vice versa.

One high-titered serum obtained by immunization with mixtures of rabbit kidney and streptococci was absorbed with rabbit kidney broth. After absorption this serum gave no serologic reaction with broth of rabbit kidney but still reacted strongly with extract of rabbit kidney.

A few rabbits which showed antibodies to kidney in the serum were killed and their serum was

TABLE 2—*Absorption Experiments. Titers of Renal Antibodies in Terms of the Highest Dilution of Serum Yielding Agglutination.*

Serum	Unabsorbed					Absorbed with						
	Kidney	Liver	Brain	Red Blood Cells	Serum	Liver		Serum				
						Liver	Kidney	Serum	Kidney	Liver	Brain	Red Blood Cells
1	320	160			40	0	320	0	320			
2	160	80			20	0	160	0	80			
3	320	80				0	320					
4	160	40	20	40	160			0*	160*	40*	0*	0*
5	320	40	20	40	160			0*	160*	40*	0*	0*
6	320	80	20	40	80	0	320	0*	320*	80*	0*	0*

\* The serums were absorbed with serum and red blood cells.

of 20 per cent kidney emulsion, the actual dilution in terms of renal substance would be 1 200 to 1 800.

#### SPECIFICITY OF THE ANTIBODIES TO KIDNEY DEVELOPED IN RABBITS

Some serums of moderate and higher titers were tested serologically by means of the collodion technic with respect to organ specificity with antigens such as serum, red blood cells and brain of the rabbit. In all cases the reactions with these antigens were considerably weaker than those with kidney as an antigen. Frequently no reaction at all was noted with serum or brain. The reactions with liver usually were more pronounced.

A few absorption experiments were attempted with rabbit liver and rabbit serum, the results of which are given in table 2. From this table it is evident that the antibodies reacting with tissues other than kidney could be removed by absorption with these tissues without appreciably impairing the titers of the antibodies reacting with kidney.

tested with their own kidney as an antigen. The same or a stronger reaction than that with kidney of normal rabbits was noted.

#### PRODUCTION OF ANTIBODIES TO KIDNEY IN RATS

Approximately 60 rats were immunized intraperitoneally with mixtures of perfused rat kidney and beta hemolytic streptococci which had been killed by heat or ether, the same strains of organisms, proportions of components of the admixtures and schedules of immunization as with rabbits were used. The doses varied between 0.1 and 1.0 cc. Blood was taken by syringe from the tail vein.

About 50 per cent of the immunized rats showed development of antibodies to kidney when tested serologically with collodion particles sensitized with extract of rat kidney. The titers lay between 1 20 and 1 1,280. It was observed on repeated bleeding after immunization that in rats the appearance of antibodies to kidney varied over a relatively long period, from about four days to more than three weeks after the last injection. A considerable proportion of the animals therefore may have had such antibodies in the blood at another time than when tested.

As in the case of rabbits, it was found in an additional large number of rats that preparations consisting of streptococci grown on rat kidney had only slight anti-

genicity with respect to the formation of antibodies to kidney

Control rats treated with plain rat kidney failed to show evidence of the production of antibodies to kidney, whereas controls treated with killed or living streptococci occasionally showed some, though very slight, serologic reaction with rat kidney

#### COMMENT

The results presented provide evidence that streptococci are able in some way to confer antigenicity on renal material which ordinarily is not antigenic in the same species. The underlying mechanism might most easily be explained by the conception of haptens (represented in the kidney) being attached to a "carrier" of protein nature (streptococcus or some of its substances). Such combinations when injected lead to the formation of antibodies—not only to the streptococcus but also, and independently from the latter, to kidney of the same species. Such antibodies to normal kidney of the same species have been demonstrated in the present experiment by several serologic methods: agglutination of collodion particles sensitized with extract of kidney, agglutination of suspensions of renal cells and cell fragments, and in a few instances also by precipitation of extract of kidney by the serum. The antibodies determined by the collodion method also were found to react serologically with kidney from the same animal which furnished the serum.

The conception of the hapten mechanism is supported by the observation that other antigens such as staphylococcus toxin, also can act as a carrier for the kidney material, rendering it antigenic. It seems likely that haptens of non-protein character were involved in the experiments which consisted of the immunization of rabbits with rabbit kidney broth plus streptococci. However, as shown by the absorption experiment mentioned on page 151 renal materials which

are not heat stable and which presumably are of protein nature or are nonprotein substances attached to protein also are rendered highly antigenic by the addition of streptococci.

Some results of the present studies seem to indicate that with the immunizing procedures employed two, and possibly more, different antibodies reacting with kidney *in vitro* are formed.

The renal lesions obtained in rabbits and particularly in rats are to be described in a later paper.

With respect to the genesis of glomerulonephritis in man, as already suggested by Schwentker and Comploier,<sup>2b</sup> streptococci or their substances could easily be conceived as the cause of a slight toxic damage of the kidneys during the height of a streptococcal infection, perhaps demonstrated clinically by the albuminuria and other urinary symptoms which are noted so frequently in cases of streptococcal infection. Undoubtedly, material from such damaged renal tissue could enter the blood stream during the process of reparation, perhaps still attached to the toxin which had acted on it. On reaching the sites of the formation of antibodies this complex would act as an antigen. Antibodies to kidney would be formed which, if they reach a sufficiently high level, would precipitate glomerulonephritis by their specific reaction with the hapten represented in the kidney.

#### SUMMARY

Evidence is presented by means of experiments in rabbits and rats that group A beta hemolytic streptococci are able in some way to render renal material antigenic in the same species. The antibodies to kidney produced by immunization with combinations of streptococci and homologous kidney were demonstrated by several serologic methods *in vitro*.



# CYSTS AND CYSTIC TUMORS OF THE MEDIASTINUM

T C LAIPPLY, MD

CLEVELAND

The mediastinum is the site of a large variety of cysts and cystic tumors, and the following classification is offered

Type of Tumor	Derivation
I Congenital	Ectoderm
1 Epidermoid cyst	Ectoderm and mesoderm
2 Dermoid cyst	Ectoderm, endoderm and mesoderm
3 Teratoma	Mesoderm
4 Pericardial celomic cyst	Mesoderm
5 Bronchial cyst	Entoderm and mesoderm
6 Esophageal cyst	Entoderm and mesoderm
7 Gastroenteric cyst	
(a) Gastric	Entoderm and mesoderm
(b) Enteric	Entoderm and mesoderm
8 Cystic lymphangioma	Mesoderm
II Acquired	
1 Parasitic cyst, caused by <i>Taenia echinococcus</i>	
2 Neoplastic cyst, due to degeneration of a solid tumor	
3 Cystic hematoma, resulting from degeneration of hematoma	

Of considerable interest are those thought to be congenital. In this paper the cases of 3 patients with such mediastinal tumors are reported, the genesis of the various types is discussed, and a review of the literature is presented

## EPIDERMOID CYST, DERMOID CYST AND TERATOMA

The epidermoid cyst, the dermoid cyst and the teratoma make up the largest group of intra-thoracic congenital cysts and tumors. Although not rare, they are uncommon, as indicated by Hare's<sup>1</sup> review. Of 288 mediastinal tumors (including metastatic growths) he found only 11 to be of this congenital type.

In 1825 Gordon<sup>2</sup> reported the first authentic case of congenital mediastinal dermoid cyst, containing hair, sebaceous material, a rudimentary mandible and six teeth. A little more than one hundred years later, in 1933, Hedblom<sup>3</sup> collected reports of 185 cases of epidermoid cyst, dermoid

cyst and teratoma from the literature and recorded 6 cases of his own, making a total of 191. A review of the subsequent literature up to January 1944 reveals 52 additional verified cases.<sup>4</sup> To these are added a subsequently reported instance of cancerous teratoma<sup>5</sup> and case 1 of this

4 (a) Harrington, S. W. *J Thoracic Surg* **3** 50, 1933, **7** 191, 1937. (b) Pasman, R. E., and Pepe, C. J. *Rev Asoc med argent* **47** 2992, 1933. (c) Lambret, O. *Echo med du Nord* **37** 445, 1933. (d) Bevan, A. D. *S Clin North America* **13** 1165, 1933. (e) Hablutzal, C. *Schweiz med Wchnschr* **63** 1308, 1933. (f) Kantrowitz, A. R. *Am J Path* **10** 531, 1934. (g) Hammarskjold, B. *Acta radiol* **15** 210, 1934. (h) Divis, J. *Časop lek česk* **74** 18, 1934. (i) de Castro, J. R., and Parreira, H. *An de med int* **3** 935, 1934. (j) Reddingius, T. *Nederl tijdschr v geneesk* **78** 5334, 1934. (k) Cisneros, R. *Bol y trab, Soc de cir de Buenos Aires* **19** 452, 1935. (l) Houghton, J. D. *Am J Path* **12** 349, 1936. (m) Piechaud, F., Poinot, J., and Bordenave, J. *Bull et mem Soc med et chir de Bordeaux*, 1936, p. 56. (n) Moir, P. J. *Brit M J* **1** 463, 1936. (o) Somolinos, G. *Arch cardiol y hemat* **17** 1752, 1936. (p) Stanbury, W. S., and Oille, W. A. *J Tech Methods* **16** 52, 1936. (q) Schenck, S. B. *M Rec* **144** 276, 1936. (r) Jellen, J., and Fischer, W. E. *Am J Dis Child* **51** 1397, 1936. (s) Fox, J. P., and Hospers, C. A. *Am J Cancer* **28** 273, 1936. (t) Leuret, E., Piechaud, F., and Delachaud, P. *Rev de med, Paris* **53** 61, 1936. (u) Lanza, G. *Arch per le sc med* **63** 71, 1937. (v) Fanano, V. *Riv di pat e clin d tuberc* **11** 453, 1937. (w) Wheatley, G. M. *Am J Dis Child* **54** 1057, 1937. (x) Smith, E. V., and Mills, R. G. *J Thoracic Surg* **7** 338, 1938. (y) Rudenko, O. M. *Vrach delo* **20** 511, 1938. (z) Kuzma, W. *Polski pizegl radiol* **13** 145, 1938. (a') Becker, B. J. P. *South African M J* **13** 659, 1939. (b') Doran, W. T., and Lester, C. W. *J Thoracic Surg* **8** 309, 1939. (c') Heuer, G. J., and Andrus, W. D. *Am J Surg* **50** 143, 1940. (d') Gray, H. K., and Woodruff, R. *Minnesota Med* **23** 781, 1940. (e') Aguilar, H. D. *Publ d centro de invest fisiol* **4** 309, 1940. (f') van Joost, C. R. N. F., and Kopp, J. G. *Geneesk tijdschr v Nederl-Indie* **81** 969, 1941. (g') Cabot Case 27052, *New England J Med* **224** 207, 1941. (h') Amorim, A. *Bol coll brasil de cirurgiões* **17** 17, 1942. (i') Beck, C. S. *Ann Surg* **116** 161, 1942. (j') Friedrich, R. *Zentralbl f Chir* **69** 479, 1942. (k') Carlson, H. A. *J Thoracic Surg* **12** 376, 1943. (l') Gebauer, P. W. *ibid* **12** 458, 1943. (m') Sarroca, J. L. *Bol Soc cir d Uruguay* **14** 52, 1943. (n') Neuhoof, H. J. *Mt Sinai Hosp* **10** 402, 1943. (o') Gravano, L., Itoiz, O. A., and Bianchetti, S. L. *Rev Asoc med argent* **57** 878, 1943.

5 Laipply, T. C., and Shipley, R. A. Extragenital Chorion-Epithelioma in the Male, *Am J Path*, to be published

From the Institute of Pathology, Western Reserve University and the University Hospitals of Cleveland

1 Hare, H. A. *The Pathology, Clinical History and Diagnosis of Affections of the Mediastinum Other Than Those of the Heart and Aorta*, Philadelphia, P. Blakiston's Son & Co., 1889, cited by Houghton.<sup>41</sup>

2 Gordon, J. A. *Med-Chir Tr*, London **13** 12, 1827, cited by Hedblom.<sup>3</sup>

3 Hedblom, C. A. *J Thoracic Surg* **3** 22, 1933



paper, making a total of 245 cases of epidermoid cyst, dermoid cyst and teratoma

*Origin*—Many hypotheses have been advanced to explain the origin of dermoid and teratoid tumors, but the exact derivation of the tissues of which they are composed has never been definitely determined. Two fundamentally different hypotheses are the monogerminal and the bigerminal.

The monogerminal hypothesis implies that in every case the tumor develops from one embryo. One of the proponents of this theory suggested origin by invagination of the ectoderm at the time of the closure of the primitive wall of the thorax. This concept, however, was based on the incorrect belief that these cystic tumors were made up only of tegmental structures. Other suggestions include origination from branchial clefts, thyroid gland and bronchi but fail to explain the complex tumors with three embryonal derivatives. Perhaps the most satisfactory hypothesis of the monogerminal type is the one indicating development from totipotent cells in embryonal rests of the urogenital fold.

The bigerminal hypothesis maintains that there is a second independent embryonal anlage which never reaches normal body form or structure, remaining as a parasitic fetus in fetu.

Some writers contend that the complex teratoma is of bigerminal origin, while the more simple dermoid and epidermoid cysts are explained by the monogerminal hypothesis.

Even though these theories offer some explanation for the development of the tumor of this group, they do not elucidate the fact that many of these tumors remain dormant or insignificant in size for years before they enlarge and produce symptoms.

*Sex and Age of Patients*—Of the 245 patients whose cases are reported in the literature, 106 were males and 118 were females, in 21 cases the sex was not stated.

The incidence with respect to the age at the time when the diagnosis was established by operation or autopsy may be stated as shown in table 1.

TABLE 1—Age Incidence

Age Group	Number	Percentage
Under 12 years	31	12.7
12-16 years	19	7.8
17-20 years	34	13.8
21-30 years	87	35.5
31-40 years	30	12.2
41-50 years	13	5.3
51-60 years	9	3.7
61-70 years	1	0.4
Age not stated	21	8.6

Note: Hedblom.<sup>3</sup> For convenience the age groups are those used by

The youngest patient was a stillborn infant, the oldest, a 62 year old woman. As indicated in the tabulation, these tumors are most common in the younger age groups, 69.8 per cent occurring in those less than 31 years of age.

*Structures*—Hedblom's classification divides the congenital intrathoracic tumors into three groups: epidermoids, dermoids and teratomas.

The epidermoids, containing only derivatives of ectoderm, are cysts lined with stratified squamous epithelium. Their walls are made up of dense fibrous tissue with or without glands of ectodermal origin. They are filled with clear or milky fluid or with a gelatinous or pasty material, frequently mixed with hair.

The dermoids are also cysts and have tissues of mesodermal as well as ectodermal origin. Thus, in addition to hair, epithelium and glands, there are cartilage, bone, teeth, smooth or striated muscle and other structures.

The teratoma on microscopic examination shows tissues derived in part from entoderm, as well as from ectoderm and mesoderm. It is usually more solid than the other congenital tumors and contains varying combinations of tissues derived from the digestive tract and its associated glands and from the respiratory tract, the thyroid gland and the thymus.

The 245 cases in the epidermoid, dermoid and teratoma groups are classified as follows:

Epidermoid group	
Microscopically examined	48
Not microscopically examined	59
Dermoid group	
Microscopically examined	47
Not microscopically examined	21
Teratoma group	
Microscopically examined	59
Not classified	11

Two hundred and seventeen (88.6 per cent) of the growths were benign, and 28 (11.4 per cent) were cancerous.

*Symptoms and Signs*—The two most common symptoms were cough and pain in the chest. Cough, which was present in 115 patients, was productive of more or less purulent sputum in 49. Hemoptysis was noted in 35, and hair was coughed up by 33 of the patients. Pain in the chest with or without radiation to the shoulders or the arms was a prominent symptom in 78. Other symptoms included dyspnea in 24, marked loss of weight in 15, palpitation in 8, hoarseness in 8, dysphagia in 6 and weakness in 4.

There was dullness or flatness to percussion in 97, bulging of the chest in 38, cyanosis in 20, edema of the arms or the neck in 13, fever in 14,

displacement of the heart in 11, enlargement of cervical and thoracic veins in 12, signs of pleurisy with or without effusion in 24, signs of empyema in 10 and signs of pericarditis in 9

As indicated by the age incidence, there was frequently a latent period of several years during which the tumor increased in size sufficiently to cause recognized symptoms, and in the majority of instances the onset of symptoms was insidious. The duration of the symptoms up to the time at which the diagnosis was established by operation or autopsy in 151 patients was less than six months in 45, six months to one year in 26, one to five years in 53, five to ten years in 21, ten to twenty years in 5 and thirty to forty years in 1.

*Prognosis and Treatment*—Without treatment the prognosis varies with the size, the location and the rate of growth of the tumor. Some of the tumors remained small, produced only minor or no symptoms and were incidental unexpected observations at autopsy.

Roentgen treatment was of no value with the benign tumors and had no lasting effect in the cancerous ones. Death occurred in all patients with cancerous tumors so far as the result was stated.

The prognosis with surgical treatment also varies with the size and the location of the tumor. The first case in which a patient was operated on was that reported by Pohn<sup>6</sup> in 1871, he drained a dermoid that presented in the neck. Complete extirpation of such a tumor was first successfully accomplished in 1893, by Bastianelli.<sup>7</sup> In recent years the development of thoracic surgery has led to successful surgical treatment in many cases.

The results of surgical treatment in the cases reported in the literature are indicated in table 2.

TABLE 2—Results of Surgical Treatment

Treatment	Cases	Patients Cured	Patients Improved	Patients Not Improved	Patients Who Died	Cases in Which Result Is Not Stated
Drainage	22	0	11	1	8	2
Marsupialization and/or partial excision	28	10	15	0	3	0
Complete excision	76	55	7	0	12	2

Of the 126 patients subjected to surgical treatment, 65 (or 51.5 per cent) were cured, 33 (or 26.2 per cent) were improved, and 23 (or 18.3

per cent) died. The most common postoperative complication was a persistent bronchopleural fistula. Complete extirpation of the tumor cured 55 (or 72.4 per cent) of 76 patients so treated. Thus, the treatment of choice is radical excision. This is best accomplished before pressure symptoms or infection have supervened.

#### PERICARDIAL CELOMIC CYSTS

The pericardial cysts are thought to result from an anomalous development of the pericardium, which is formed by the fusion of multiple disconnected lacunae. If one of the lacunar cavities failed to merge with the others, it could persist and develop into a pericardial celomic cyst. If, on the other hand, the rate of development of the primitive cavities was unequal, an unusually large lacunar space in continuity with the others could result in a congenital diverticulum of the pericardium.

The pericardial celomic cysts have thin, loose or dense fibrous connective tissue walls and are lined with a single layer of flattened endothelial or mesothelial cells. They are situated in the anterior mediastinum in contact with the anterior thoracic wall, the parietal pericardium and sometimes the diaphragm and one of the lungs.

Cases of cysts of this type have been reported by Dufour and Mourret,<sup>8</sup> Pickhardt<sup>9</sup> and Lambert.<sup>10</sup> The cyst in the case reported by Dufour and Mourret was an incidental observation at autopsy in an 86 year old woman dying of cerebral infarction. Pickhardt reported the case of a 53 year old woman who complained of persistent thoracic pain. At operation a cyst of the anterior mediastinum was encountered and excised, with complete relief of the patient's symptom. Lambert reported 2 cases of cyst in the anterior mediastinum. In each the cyst was asymptomatic and was discovered on roentgen examination in the course of routine studies. In each instance it was removed and the patient had an uneventful postoperative course.

#### BRONCHIAL CYSTS

Bronchial cysts have also been referred to as bronchogenic, ciliated columnar epithelial and reduplication cysts of the respiratory tract. The theoretic origins include a pinching off of a diverticulum of the foregut in the region of the tracheal bud, a secondary budding of the tracheal bud itself and an abnormal division of the

6 Pohn, H. Beschreibung eines Falles von Dermoid-cyste des Mediastinum anticum, Inaug. Dissert., Berlin, G. Lange, 1871, cited by Hedblom.<sup>3</sup>

7 Bastianelli, R. Riforma med., May 20, 1893, cited by Hedblom.<sup>3</sup>

8 Dufour, H., and Mourret. Bull. et mem. Soc. med. d. hôp. de Paris 53:1482, 1929, cited by Lambert.<sup>10</sup>

9 Pickhardt, O. C. Ann. Surg. 99:814, 1934.

10 Lambert, A. V. S. J. Thoracic Surg. 10:1, 1940.

tracheobronchial tree at a later stage of development

*Origin*—A developmental abnormality seems probable because of the way in which the trachea and the bronchi are formed from the primitive foregut. In the 2.5 mm embryo the trachea and lung bud are seen as a pear-shaped mass attached to the ventral border of the esophagus. In the 4 mm embryo the bud begins to bifurcate and the respiratory organs are represented by the laryngeal groove, the tubular trachea and two lung buds or primary bronchi. At this time the cavity in the primitive respiratory organs is still continuous with that of the trachea. The trachea becomes separated from the esophagus by the downward growth of the lung buds and the upward extension of the notch between the lungs and the esophagus. The fusion of the lateral walls to form the tracheoesophageal septum begins from below. It would seem that at this time a pinching off of a small bud or diverticulum of the foregut might occur. This could subsequently be carried caudally by the downward growth of the lungs to the mediastinum. Such a diverticulum would contain entoderm and mesoderm destined to become a part of the trachea, the bronchi, the esophagus, the stomach or the intestine. This theory offers an explanation for bronchial, esophageal, gastric and enteric cysts of the mediastinum.

*Structure and Location*—Bronchial cysts may contain any or all of the tissues that are normally present in the trachea and the bronchi. Typically they are lined with ciliated pseudostratified columnar epithelium (fig 2). In some areas the cilia and even the epithelium may be absent. Their walls consist principally of fibrous connective tissue and may contain mucous glands, hyaline cartilage (fig 3), smooth muscle, which is sometimes arranged in layers, nerve trunks and elastic fibers. Some of the cysts considered to be bronchogenic are in part lined with stratified squamous epithelium. They vary in size and usually contain clear viscid fluid or gelatinous material. Although they may occur at any site along the tracheobronchial tree, they are most common in the posterior part of the superior mediastinum, particularly in the region of the tracheal bifurcation. The cysts had unusual locations in one of the cases reported by von Westenryk<sup>11</sup> and in that described by Stoeckenius.<sup>12</sup> In both instances the ciliated epithelial cysts were small and unexpected discoveries at autopsy. Von Westenryk's case was that of a 38 year old

man who died with right hemiplegia. A small cyst was present in the submucosa of the lower part of the esophagus. Stoeckenius described a similar small cyst within the heart at the upper end of the posterior papillary muscle of the left ventricle. This was an incidental observation in a 45 year old man who died of pneumonia after gastrectomy for carcinoma.

The lumens of the cysts typically do not communicate with the trachea or the bronchi. A communication with the trachea was observed in the 5 cases reported by Chiari.<sup>13</sup> In these instances the anomalies were tracheal diverticula and not true cysts.

*Number*—Thirty-four cases<sup>14</sup> of mediastinal cyst of bronchial type had been reported in the literature up to January 1944. To these is added case 2 of this paper, making a total of 35.

*Sex and Age of Patients*—Of the 35 patients, 19 were males, and 14 were females, in 2 instances the sex was not indicated.

The distribution of the cases with regard to the age at the time when the diagnosis was confirmed by operation or autopsy is shown in table 3.

Other probable cases have been reported by Harrington,<sup>15</sup> Walzel,<sup>16</sup> Ellis<sup>17</sup> and Stoeckel.<sup>18</sup>

13 Chiari, H. Beitr. z. path. Anat. u. z. allg. Path. **5** 329, 1888.

14 (a) Stilling, H. Virchows Arch. f. path. Anat. **114** 557, 1888. (b) Joel, J. *ibid.* **122** 381, 1890. (c) Zahn, F. W. *ibid.* **143** 170, 1896. (d) von Rau, F. *ibid.* **153** 26, 1898. (e) von Wyss, H. *ibid.* **51** 143, 1870. (f) Eppinger. Pathologische Anatomie des Larynx und der Trachea, Berlin, A. Hirschwald, 1880, p. 256, cited by von Westenryk.<sup>11</sup> (g) Herrmann. Prag. med. Wchnschr. **15** 146, 1890, cited by von Westenryk.<sup>11</sup> (h) Fletcher, H. M. Tr. Path. Soc. London **48** 249, 1896-1897. (i) von Springer, C. Prag. med. Wchnschr. **23** 393, 1898. (j) Wechsberg, F. Zentralbl. f. allg. Path. u. path. Anat. **11** 593, 1900. (k) Bert, P., and Fischer, B. Frankfurt. Ztschr. f. Path. **6** 26, 1911. (l) Blackader, A. D., and Evans, D. J. Am. J. Dis. Child. **51** 1126, 1936. (m) Gold, E. Beitr. z. path. Anat. u. z. allg. Path. **68** 278, 1921. (n) Nossen, H. Deutsche med. Wchnschr. **51** 1151, 1925. (o) Ehlers, H. W. E. Deutsche Ztschr. f. Chir. **215** 189, 1928. (p) Mixer, C. G., and Clifford, S. H. Ann. Surg. **90** 714, 1929. (q) Alford, J. E. Arch. Path. **23** 296, 1937. (r) Rizzi, I. Arch. ital. di anat. e istol. pat. **8** 689, 1938, cited by Carlson.<sup>14v</sup> (s) Johnston, L. M. Am. J. Dis. Child. **56** 313, 1938. (t) Congenital Cyst of Mediastinum, Cabot Case 26291. New England J. Med. **223** 105, 1940. (u) Wyllie, W. G., and Pilcher, R. S. Arch. Dis. Childhood **18** 34, 1943. (v) Carlson, H. A. J. Thoracic Surg. **12** 376, 1943. (w) Adams, W. E., and Thornton, T. F. *ibid.* **12** 503, 1943. (x) Heuer and Andrus.<sup>14e</sup> (y) Stoeckenius.<sup>12</sup> (z) von Westenryk.<sup>11</sup>

15 Harrington, S. W. Arch. Surg. **19** 1679, 1929.

16 Walzel, P. Beitr. z. klin. Chir. **158** 654, 1933.

17 Ellis, W. B. Proc. Roy. Soc. Med. **28** 666, 1935.

18 Stoeckel, K. H. Zentralbl. f. Gynak. **59** 2178, 1935.

11 von Westenryk. Prag. med. Wchnschr. **25** 373, 1900.

12 Stoeckenius, W. Zentralbl. f. Herz- u. Gefasskrankh. **11** 73 and 89, 1919, cited by Ehlers.<sup>14o</sup>

In these instances the structure was suggestive but not absolutely characteristic of a bronchial cyst

*Symptoms and Signs*—Because of their frequent occurrence in the region of the trachea and the main stem bronchi, bronchial cysts of small size may produce signs of obstruction in the air passages early in life. Not infrequently, how-

ever, as in 16 of the 35 cases, the cysts remain quiescent, produce no symptoms and are discovered at autopsy. The cysts were incidentally observed at autopsy in the cases of the 60 year old woman and the 66 year old man reported by Beit and Fischei<sup>141</sup> and by Rizzi,<sup>142</sup> respectively, as well as in 14 other instances. Most commonly, however, the symptoms appear during the first

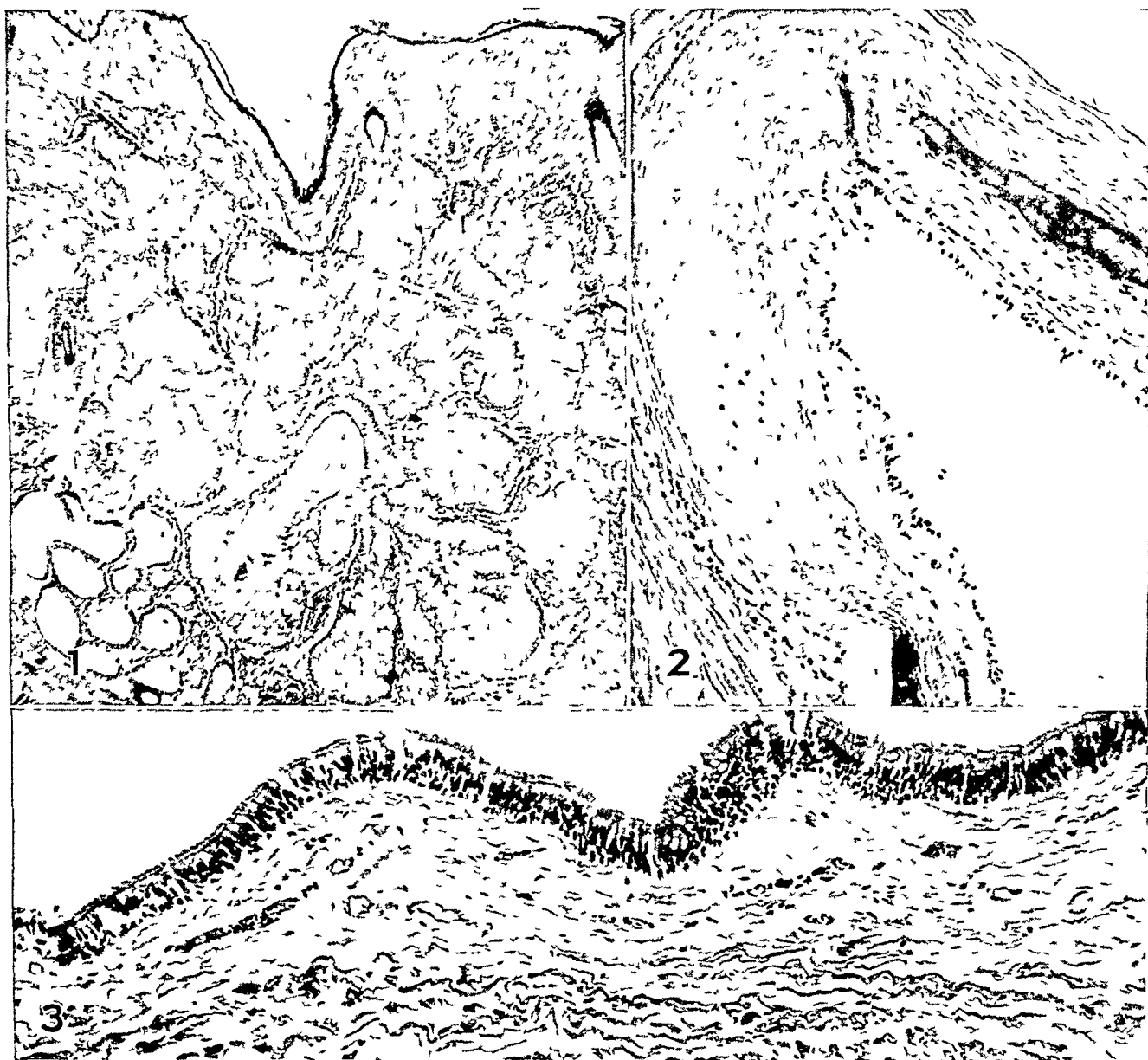


Fig 1 (case 1)—Stratified squamous epithelium lines the cyst. Hair follicles, apocrine glands and many sebaceous glands are in adjacent connective tissue. Hematoxylin and eosin,  $\times 385$

Fig 2 (case 2)—Typical bronchial or ciliated pseudostratified columnar epithelium lines the cyst. Hematoxylin and eosin,  $\times 158$

Fig 3 (case 2)—Cartilage and bone are present in the cyst wall. The inner part of the lining ciliated pseudostratified columnar epithelium is desquamated. Hematoxylin and eosin,  $\times 117$

TABLE 3—Age Incidence

Age Group	Number
Stillborn	1
Less than 1 year	9
1 to 9 years	3
10 to 19 years	1
20 to 29 years	3
30 to 39 years	7
40 to 49 years	4
50 to 59 years	2
60 to 69 years	2
Age not stated	3

few months of life. It is even possible that the infant with such a cyst may be stillborn because of it. Cyanosis, dysphagia (difficulty in feeding) and cough are also frequent.

Examination may reveal signs of pneumonia or atelectasis of the lung. Dulness to percussion, cardiac displacement, bulging of the thorax and scoliosis may be noted. The diagnosis during

life may be difficult or impossible to reach because of the small size and the posterior position of the cyst. Such cysts are difficult to visualize by roentgen rays, especially when, as is commonly true, they are surrounded by atelectatic or consolidated pulmonary tissue. Bronchography and especially bronchoscopy should be helpful in diagnosis by demonstrating the narrowing of the trachea or of the bronchi due to extrinsic pressure.

*Treatment*—As with other mediastinal cysts and tumors, complete excision is the treatment of choice. In 6 of the reported cases the patient was operated on. The cyst in 4 was completely removed, and the patient was cured, in 2 cases death resulted from the operation.

#### ESOPHAGEAL CYSTS

Esophageal cysts would have structures simulating the normal esophagus. It seems likely from the developmental standpoint that such cysts could occur in the mediastinum. Nevertheless, none of those reported in the literature has been so classified.

Esophageal cysts would be lined with stratified squamous epithelium and would have fibrous connective tissue walls, possibly containing mucous glands and layers of smooth muscle. They should be differentiated from epidermoids and dermoids, which do not closely simulate the structure of the esophagus.

Several of the cases reported in the literature have had tissues like those of two different organs. Such mixed cysts are easily explained as developmental abnormalities. The following mediastinal cysts of mixed type have been reported: gastric and esophageal (Carlson<sup>4k</sup>, Stahelin and Burckhardt<sup>19</sup>, Smith<sup>20</sup>), bronchial and esophageal (Adams and Thornton<sup>14c</sup>) and tracheal and esophageal (Guillery<sup>21</sup>). The predominance of either gastric or bronchial elements has resulted in their being classed as either gastric or bronchial cysts.

In the case reported by Melchoir<sup>22</sup> there was a mediastinal cyst with a microscopic structure much like that of the normal esophagus. The lining epithelium was almost entirely of the stratified squamous type and the fibrous connective tissue wall contained mucous glands. A single focus of columnar epithelium was noted, but the presence of cilia could not be established with certainty. The author considered this to be

a bronchial cyst, the original bronchial epithelium having undergone metaplasia to the stratified squamous type. Such an occurrence certainly cannot be denied but it also seems possible that the pinching off of a portion of foregut made up of tissue destined to be esophageal could have occurred.

#### GASTROENTERIC CYSTS

This group consists of cysts with structures simulating normal stomach and intestine. Their origin has been ascribed to the pinching off of a bud or a diverticulum of the embryonic foregut, to an intrathoracic vestige of the omphalomesenteric (vitelline) duct and to proliferation of an entodermal germ cell of the esophagus.

Occasionally the gastric and enteric cysts are structurally so similar that careful examination of several sections from different portions of the wall is necessary for proper classification. As indicated in an earlier section, some of the cysts in this group also contain esophageal, tracheal or bronchial elements.

#### GASTRIC CYSTS

*Structure and Location*—The microscopic features of the gastric or gastrogenic cysts are like those of normal stomach. All of the coats of normal stomach may be present: the mucosa, the muscularis mucosae, the submucosa, the muscularis and the serosa (fig. 4). In the lining mucosa there are deep branching glands of the gastric type (fig. 5), and in some instances both parietal and chief cells may be present. Nerve trunks and groups of ganglion cells occur in the muscularis. The outer surface is usually in part covered with pleura. The continuity of the lining mucosa may be interrupted, and lesions simulating chronic peptic ulcers were noted in the mediastinal gastric cysts described by Boss<sup>23</sup> and by Seydl<sup>24</sup>. In both instances the ulcers had penetrated into the adjacent lung, resulting in intrapulmonary hemorrhage and hemoptysis.

These cysts are usually situated paravertebrally in the posterior mediastinum, behind the trachea and the esophagus. They vary considerably in size, are usually unilocular and contain from a few to several hundred cubic centimeters of clear amber, turbid or sanguineous viscid fluid. This fluid may be neutral or strongly acid in reaction. The cysts are frequently firmly adherent to adjacent structures, especially the esophagus and the lung. Attachment to or extension into the diaphragm and erosion of vertebrae

<sup>19</sup> Stahelin and Burckhardt. Arch f Verdaunungskr 15 584, 1909, cited by Seydl<sup>24</sup>

<sup>20</sup> Smith, R. E. Guy's Hosp Rep 80 466, 1930

<sup>21</sup> Guillery, H. Zentralbl f allg Path u path Anat 69 49, 1937

<sup>22</sup> Melchoir, E. Zentralbl f Chir 56 2626 1929

<sup>23</sup> Boss, C. Virchows Arch f path Anat 300 166, 1937

<sup>24</sup> Seydl, G. N. Frankfurt Ztschr f Path 52 346, 1938

and ribs were noted in the cases reported by Mixer and Clifford<sup>14p</sup>

*Number*—Twelve cases<sup>25</sup> of mediastinal gastric cyst had been recorded in the literature up to Jan 1, 1944. To these are added the case recently reported by Olken<sup>26</sup> and case 3 of this paper, making a total of 14 such cases.

*Sex and Age of Patients*—Of the 14 patients, 8 were males, and 5 were females, in 1 case the sex was not stated.

All the patients were infants or children, as indicated in the following tabulation:

	Number
Less than 1 year	7
1 to 2 years	4
2 to 10 years	3

*Symptoms and Signs*—The symptoms, like those of other similarly located cysts, are largely due to compression of the trachea or the bronchi and include dyspnea, cyanosis, dysphagia (difficulty in feeding) and occasionally, even in infants,<sup>24</sup> hemoptysis.

The physical findings may be primarily those of pulmonary atelectasis or pneumonitis. Cardiac displacement, scoliosis and bulging of the chest also occur. Although the contents may be neutral in reaction, the aspiration of strongly acid fluid should be helpful in diagnosis.

*Treatment*—In 6 of the 14 cases complete surgical excision was attempted. In 3 complete excision cured the patient, in the other 3 death occurred during or soon after the operation.

#### ENTERIC CYSTS

These have also been called enterogenous cysts, *Enterokystome*, *Darmkystome* and *Darmzysten* (intestinal cysts). They are similar to the gastric cysts except that their microscopic structure simulates that of the normal intestine.

*Number*—Three cases with such mediastinal cysts<sup>27</sup> had been recorded in the literature up to Jan 1, 1944. Two other probable cases of enteric cyst have been reported.<sup>28</sup> The absence of mucosa in the cyst in each case prevents exact classifica-

tion. Both cases should, therefore, be designated as cases of gastroenteric cyst of the mediastinum.

*Sex and Age of Patients*—Four of the 5 patients were males, and 1 was a female. Three were stillborn, 1 was 15 months old and 1 was 4½ months of age.

Intra-abdominal cysts of similar type were present in 3 of the 5 cases of enteric or probable enteric cyst. The concomitant occurrence of such cysts in the abdomen and the thorax suggests the possibility of their originating from the omphalomesenteric duct.

#### CYSTIC LYMPHANGIOMA

Cystic lymphangioma (cystic hygroma) is probably of congenital origin and is among the less common of the mediastinal cystic tumors. It is characterized by multiple variable-sized cystic spaces, lined with a single layer of endothelium and containing gelatinous material or fluid that is clear and colorless or brown. The fibrous connective tissue between the spaces not infrequently contains scattered smooth muscle fibers, foci of lymphocytes, fat cells and cholesterol crystals. The lesion is not encapsulated and has ill defined borders, usually intimately associated with the great vessels and surrounding structures, thus making complete excision almost impossible. Attempts at removal may result in profuse bleeding.

The mode of origin is not certain. It is suggested that a portion of the anlage for the formation of blood vessels is drawn down from the gill clefts by the descent of the pericardium. An origin from the thymus has also been considered. In some cases the mediastinal cystic lymphangioma may be the result of direct intrathoracic extension of a similar tumor of the neck.

Cases of mediastinal lymphangioma have been reported by Michaelis<sup>29</sup> and by Skinner and Hobbs<sup>30</sup>. The symptoms are not pathognomonic of this type of cyst but the onset of symptoms is probably earlier than in others.

#### REPORT OF CASES

**CASE 1**—R. P., a white woman 22 years of age, was admitted to the hospital on June 16, 1944. She had been in good health until several months prior to hospitalization, when she contracted lobar pneumonia, a roentgenogram of the chest revealed a large tumor of the mediastinum. Several roentgen treatments were given over the site of the tumor. It apparently decreased in size for a short time and then began to

<sup>29</sup> Michaelis, O. *Deutsche Ztschr f Chir* **242** 250, 1934.

<sup>30</sup> Skinner, G. F., and Hobbs, M. E. *J Thoracic Surg* **6** 98, 1936.

<sup>25</sup> Entz, B., and Orosz, D. *Frankfurt Ztschr f Path* **40** 229, 1930. Fischer, W. *Virchows Arch f path Anat* **275** 711, 1930. Poncher, H. G., and Milles, G. *Am J Dis Child* **45** 1064, 1933. Nicholls, M. F. *Brit J Surg* **29** 137, 1940. Mixer and Clifford<sup>14p</sup>. Carlson<sup>4k</sup>. Wyllie and Pilcher<sup>14u</sup>. Guillery<sup>21</sup>. Stahelin and Burckhardt<sup>19</sup>. Smith<sup>20</sup>. Boss<sup>23</sup>. Seydl<sup>24</sup>.

<sup>26</sup> Olken, H. G. *Am J Path* **20** 997, 1944.

<sup>27</sup> Roth, M. *Virchows Arch f path Anat* **86** 371, 1881. Brass, K. *Frankfurt Ztschr f Path* **50** 26, 1936. Schminke, A. *Virchows Arch f path Anat* **227** 12, 1920.

<sup>28</sup> Hennig, C. *Centralbl f Gynak* **4** 398, 1880. Black, R. A., and Benjamin, E. L. *Am J Dis Child* **51** 1126, 1936.

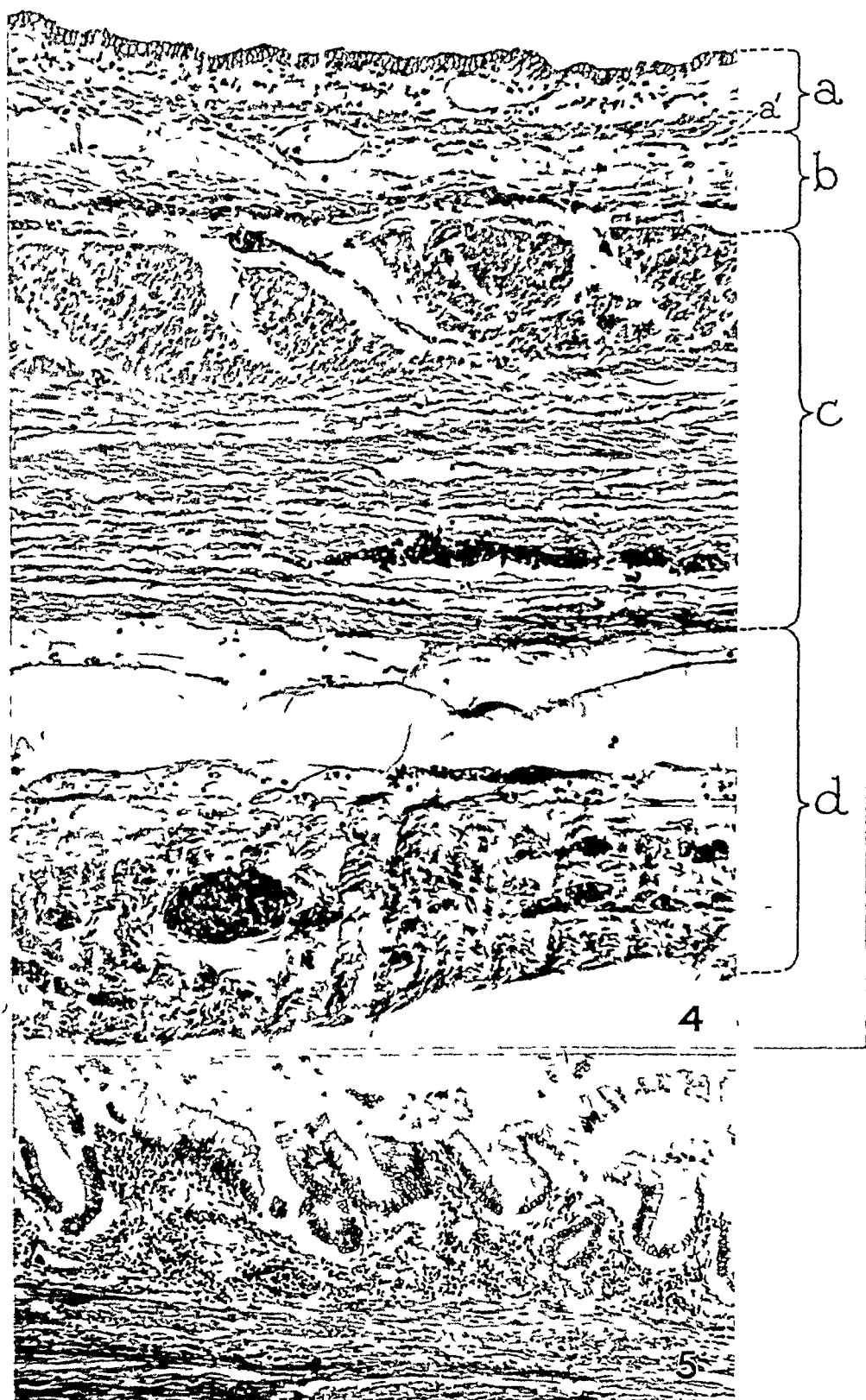


Fig 4 (case 3) —Four coats like those of the intestine are present in the cyst wall (a) mucosa, (a') muscularis mucosae, (b) submucosa, (c) muscularis (two layers), (d) serosa Hematoxylin and eosin,  $\times 165$

Fig 5 (case 3) —Glands like those of gastric mucosa are in the inner part of the cyst Hematoxylin and eosin,  $\times 102$



enlarge relatively rapidly. During the nine months subsequent to her recovery from pneumonia the patient had a persistent cough, productive of thick yellow sputum. The cough was worse when she was in the recumbent position. Blood was coughed up on two occasions. For two weeks before hospitalization she had had constant substernal pain.

On admission she was normally developed and well nourished. The body temperature was 37.8 C (100 F), the pulse rate 100, the respiratory rate 25 and the blood pressure 130 systolic and 90 diastolic. The thorax was symmetric and normally resonant, with diminished breath sounds to the right of the midline posteriorly at the level of the fourth to the sixth thoracic vertebrae. The urine was normal, the blood count showed 8,600 white cells. The hemoglobin was 75 per cent (Sahli). Roentgenograms showed a lobulated, sharply defined tumor (12 by 5 by 6 cm) along the right side of the heart. In its upper part were several molar teeth and an irregularly shaped mass of bone. A diagnosis of teratoma was made.

On her third day in the hospital the patient was operated on. The anterior portion of the right fourth rib was resected, and a large cystic tumor was seen in the anterior mediastinum, about 10 cm in diameter. The tumor was firmly adherent to the upper lobe of the right lung, from which it was dissected with considerable difficulty. The outer portion of the cyst wall could not be completely excised, but all of its inner portion and contents were removed. The lung was cut into and sutured at several sites. A drainage tube was inserted. For nine days after the operation the patient's temperature fluctuated between 38.2 and 41 C (100.7 and 105.8 F). The white blood cell count varied between 14,600 and 13,200. Over a period of eleven days following the operation 1,275,000 units of penicillin were administered. After this the patient gradually improved, and her temperature returned to normal. She was discharged on the seventeenth postoperative day.

The surgical specimen consisted of four irregularly shaped pieces of tissue, weighing 205 Gm, evidently portions of the wall of a cyst. There was a large piece of bone with two attached molar teeth, considerable grumous material in which there were many brown hairs, some gray fibrous tissue and yellow fat. Microscopic examination showed the cyst to have a stratified squamous epithelial lining. The wall consisted of fibroadipose tissue of mature type, and in its inner portion there were many hair follicles and sebaceous glands (fig 1). Attached to one part was a small piece of lung, the seat of marked acute and chronic interstitial pneumonitis.

**Diagnosis** Dermoid cyst of the anterior mediastinum.

**CASE 2**—J. M., a Negro man aged 45 years, was hospitalized because of painless jaundice and marked loss of weight. An exploratory laparotomy revealed tumor in the peripancreatic tissue, the omentum and the liver. The tissue was unusually friable, and an artery was inadvertently torn. Shortly after the profound hemorrhage which resulted, the patient went into shock and died.

**Autopsy** (Dr O. Eitzen) revealed well differentiated scirrhous adenocarcinoma of the tail of the pancreas with metastases in the liver, the peritoneum and the abdominal lymph nodes and extension into and partial obstruction of the main hepatic bile duct. There were also laceration of the gastroduodenal artery, generalized icterus and cholemic nephrosis.

An incidental finding was a cyst in the superior mediastinum, measuring 6 cm in diameter. It was attached to the superior surface of the heart, posterior and to the left of the ascending aorta. It was firmly adherent to the outer surfaces of the atriums, the

parietal pericardium, the trachea and the upper portions of the lungs. Its wall consisted of firm pale gray tissue and varied from 1 to 2 mm in thickness. The inner surface was in large part glistening and pale gray, but at some sites it was rough and yellowish brown. The contents consisted of viscid turbid dark brown fluid which solidified on fixation in 4 per cent solution of formaldehyde.

Microscopically, the cyst was lined with ciliated pseudostratified columnar epithelium. In the roughened areas the epithelium was absent and there was hemosiderin in the inner part of the wall, indicative of remote hemorrhage. The wall consisted of fibrous connective tissue in which there were hyaline cartilage, spicules of bone and groups of smooth muscle fibers (fig 3).

**Diagnosis** Bronchial cyst of the superior mediastinum.

**CASE 3**—The patient was a premature white boy with a body weight of 1,500 Gm and a length of 38 cm. In the lower thoracic and lumbar regions there was a large defect of the skin, subcutaneous tissue and dorsal portions of the vertebrae. Death occurred twenty-seven hours after birth.

**Autopsy** (Dr B. Chomet) showed a cyst in the posterior mediastinum, measuring 3 by 2.5 by 2 cm. It contained light brown fluid and had a glistening inner surface and pale gray wall, 1 mm in thickness. The cyst was immediately adjacent to but not connected with the esophagus. Microscopic examination revealed distinct coats like those of the intestine (fig 4). The outer dense fibrous connective tissue layer was like intestinal serosa. Adjacent to this was a muscular coat with an outer circular and an inner longitudinal layer. Submucosal and mucosal coats were also evident, with a thin muscularis mucosae. The lining epithelium was largely of simple columnar type, but in some regions (fig 5) there were high columnar epithelial cells and glands of a mucus-secreting type.

**Diagnosis** Gastric cyst of the posterior mediastinum, spina bifida, umbilical hernia, Meckel's diverticulum and fetal atelectasis of lungs.

## SUMMARY

The great variety of mediastinal cysts and cystic tumors may be classified as congenital (six types) and acquired (three types). A review of the literature reveals a large number, in the congenital group. Additional dermoid, bronchial and gastric cysts of the mediastinum are reported in this paper. With these there are recorded in the literature 245 epidermoids, dermoids and teratomas, 35 bronchial cysts, 14 gastric cysts and at the most 5 enteric cysts of the mediastinum. Eleven and four-tenths per cent of the epidermoids, dermoids and teratomas were cancerous. All other types of congenital mediastinal cysts were noncancerous. Such cysts and tumors may give rise to symptoms late in life but the majority have reached sufficient size to cause symptoms during the first three decades. The most common symptoms were cough, pain in the chest, dyspnea and hemoptysis. The treatment of choice is complete surgical excision—if possible prior to the development of pressure symptoms or infection.



# SOME ENDOCRINOLOGIC CONSIDERATIONS OF CANINE NEOPLASTIC DISEASES

R M MULLIGAN, M D

DENVER

The histologic study of a number of canine neoplasms observed at autopsy and in surgical specimens and some preliminary success in the production of neoplasms in dogs by means of carcinogens stimulated a review of the available literature on canine oncology in an attempt to assemble pertinent facts to form a base line for future investigation in this field. Of particular interest in the publications surveyed was much material concerned with neoplasms of endocrine glands and of the male and female genital tract. The main headings under which the various endocrinologic considerations of canine neoplastic diseases will be taken up are as follows: "Neoplasms of Endocrine Glands Associated with Endocrinic Disturbances," "Neoplasms of the Male Genital Tract," "Neoplasms of the Female Genital Tract," "Neoplasms of Other Endocrine Glands," "Infectious or Venereal Sarcoma" and "Mammary Neoplasms."

## NEOPLASMS OF ENDOCRINE GLANDS ASSOCIATED WITH ENDOCRINIC DISTURBANCES

A syndrome of feminization in male dogs associated with carcinoma of the testis and mimicked following the administration of estrogens was recently summarized<sup>1</sup> as it occurred in 6 male dogs described in the literature. These animals possessed some or most of the following features: (1) adenocarcinoma of the testis, (2) varying degrees of atrophy of the opposite testis, (3) stratified squamous epithelial metaplasia of the prostatic urethra, ducts and acini with hypertrophy of the prostate, (4) hyperplasia of the ducts and acini of the breasts with mammary enlargement, (5) swelling of the penile sheath, (6) loss of hair, (7) attraction of other male dogs, like a female dog in estrus, and (8) depression of libido. Two other cases probably related to the 6 already reviewed include case 2 of Baldoni<sup>2a</sup> and that of a cryptorchid dog described

by Krause.<sup>3</sup> Baldoni's case 2 was that of a male hound-pointer cross, about 10 years old, which in the course of nine months displayed gradual increase in the size of the right testis and later enlargement of all breasts. The right scrotal testis was as large as a fist, the left testis was small and there were tumors in the fourth and fifth breasts. Histologic examination of the surgically removed tumors indicated that the testicular neoplasm was a carcinoma (seminiferous epithelioma or seminoma of Chevassu) and that all four mammary neoplasms were adenocarcinomas. The histologic characteristics of the testicular tumor were the same as those of the carcinomas in ectopic testes described by the author in 1913 and 1923.<sup>2b</sup> Neither of these articles was obtainable for first hand perusal. Baldoni's case 1 was that of a male pointer, about 7 years old, which for seven months had a tumor of the right hindmost breast, which gradually enlarged to the size of a fist. The animal had always showed strong sexual desire, but with the appearance of the mammary tumor it had become more retiring and remained indifferent to the presence of bitches in heat. His stamina in the hunt and his general conduct were unaltered. His testes were firm and slightly tender. The excised mammary tumor was histologically a fibroadenoma. Worthy of mention in this case, according to Baldoni, was the coincidence of disappearance of strong sexual desire, an indication of testicular atrophy, with the start of the development of the mammary fibroadenoma. Baldoni stated that it had been demonstrated in man that the breasts may be stimulated after traumatic testicular atrophy (Pende, Falta), iodine intoxication (Bergess) or other types of intoxication, like hepatic cirrhosis (Silvestrini, Corda, Zanalda, Monai, Tattomi) and that sometimes after extirpation of the testes in adults the mammary glands increase in size (Tobler) and even become functional. Baldoni mentioned a Swiss bull which soon after castration showed a remarkable enlargement of the breasts and production of milk. Krause<sup>3</sup> described a male hound

From the Department of Pathology, University of Colorado School of Medicine

1 Mulligan, R M. *Am J Path* **20** 865, 1944

2 Baldoni, A. *Mem r Accad d sc d Ist di Bologna* (a) **5** 33, 1927-1928, (b) **10** 183, 1912-1913, **10** 37, 1922-1923

3 Krause, C. *Frankfurt Ztschr f Path* **41** 405, 1931

8 years old, which six years before had been submitted to orchidectomy of the left side because of excessive sexual urge. After this operation he was never known to have had coitus again. Autopsy revealed an enlarged right testis in the right iliac region. The left testis (presumably formerly scrotal) was operatively absent. Also observed were a greatly enlarged, cystic prostate, a hypertrophied urinary bladder and a thickened urethra. The anatomic diagnoses after histologic study were: alveolar carcinoma of the right testis, carcinoma of the prostate, hypertrophy of the urinary bladder, chronic pyelonephritis and atrophy of the right epididymis and ductus deferens. The anatomic description and photomicrographs of the prostate when carefully perused indicate that the true condition of this gland was stratified squamous epithelial metaplasia of the prostatic urethra, ducts and acini and not carcinoma. The hypertrophy of the urinary bladder and the chronic pyelonephritis were probably the results of prostatic enlargement and urethral obstruction. The diagnosis of carcinoma of the right intra-abdominal testis in this case is unquestionably valid from the evidence presented. Zuckerman and McKeown<sup>4</sup> reported observations on a group of 15 dogs with testicular adenocarcinoma. Five of these dogs showed concomitant squamous metaplasia of the gland system of the prostate and a sixth dog had an associated prostatic carcinoma. The authors presumed that estrogenic hormone had been elaborated by the adenocarcinoma in the 5 dogs with squamous metaplasia of the prostate, a finding indicating estrogenic stimulation. In summary, it may be said that male dogs suffering from testicular carcinoma may show certain clinical abnormalities and tissue changes, which have been detailed, the basis for these is some sort of estrogenic stimulation through a substance elaborated directly by the testicular tumor or of some substance secreted by the tumor and changed by a metabolic process into a compound with a feminizing action.

Although not strictly a neoplasm but rather a form of hyperplasia, benign prostatic hypertrophy (which commonly occurs in old male dogs as demonstrated in 22 of 37 male dogs afflicted with this lesion in the series of Goodpasture<sup>5</sup>) may be influenced to the point of regression by castration. Hobday<sup>6</sup> described 3 male dogs in

which prostatic hypertrophy was relieved by castration. The first was a toy terrier, about 4 years old, with strangury, oliguria and prostatic enlargement so severe that a catheter could not be passed. Within three weeks after castration performed after the animal had been anesthetized with chloroform, urination was free, a catheter was passed easily, the prostate was smaller, and other symptoms were absent. The second was a collie, 9 years old, with straddling gait of the hindlegs, difficulty in walking or rising, objective pain in the loins and flanks, and difficulty in starting his stream, with subsequent passage of a large amount of urine. Ten days after castration, urine was passed easily, and the formerly enlarged prostate was much smaller. The third was a Dandie Dinmount, about 11 years old, with dysuria, straddling gait and an enormous prostate, painful to pressure. Three days after castration the urinary flow was unimpeded, no relapse was observed two years later. These cases indicate that castration either breaks a chain controlled by the pituitary gland or eliminates the interstitial cells of the testes as a primary source of androgenic stimulus. The reason that castration works so well in alleviating prostatic hypertrophy in the dog as compared with orchidectomy in men afflicted with this malady may be contained in a paper by Moore,<sup>7</sup> who stated that 80 per cent of male dogs over 8 to 10 years of age show enlargement of the prostate. His opinion was that the etiologic factors may be the same in both man and the dog but that the essential lesion in prostatic enlargement in the dog is diffuse hyperplasia while that in man is nodular hyperplasia. In the experience of Zuckerman and McKeown,<sup>4</sup> diffuse hyperplasia was the rule in enlargement of the canine prostate. In the human prostate affected by benign enlargement one can demonstrate hyperplastic glandular nodules, atrophic cystic epithelial structures, mostly peripherally placed, often distended by inspissated material and involved in inflammatory changes, and proliferated fibromuscular stroma, sometimes arranged in the form of actual fibroleiomyoma and often marked by foci of chronic inflammatory cells. Because of these exaggerated anatomic abnormalities inherent in nodular hyperplasia (if not for some unknown hormonal factors) of the human prostate, it seems logical that after castration they would be less likely to regress than the rather symmetrically proliferated prostatic structures observed in the diffuse hyperplasia characterizing benign enlargement of the canine prostate.

4 Zuckerman, S, and McKeown, T. *J. Path. & Bact.* **46** 1, 1938.

5 Goodpasture, E. W. *J. M. Research* **38** 127, 1918.

6 Hobday, F. T. G. *Surgical Diseases of the Dog and Cat*, edited by J. McCunn, Baltimore, Williams & Wilkins Company, 1939, pp. 273-274.

7 Moore, R. A. *Surgery* **16** 152, 1944.

The papers cited<sup>8</sup> described endocrine changes in association with tumors of the canine male sex glands. The first discovered instance of endocrine changes in association with tumors of canine female sex glands was described by Wolfe, Cleveland and Campbell<sup>9</sup>. With the genitalia as a guide to the stage in the estrual cycle at which the gland was examined, these authors quantitatively studied the cellular composition of the anterior lobe of the pituitary gland in 79 female dogs. One animal which showed external bleeding ten days before autopsy disclosed follicular cysts of the ovaries and cystic glandular hyperplasia of the endometrium. The anterior lobe of the pituitary gland of this dog contained an increased number of acidophils. DeVita,<sup>10</sup> basing his preliminary report on the tissues and histories of 24 cases, in 5 of which histologic sections were made, described the clinical features, the ovarian and uterine pathologic changes and illustrative cases of the condition known as hyperplastic endometritis or so-called pyometra of the bitch. He also quoted Lesbouyries and Berthelon, but listed no specific reference to their work on 4 dogs with the disease which were treated with gonadotropic hormone and oophorectomy. DeVita stated that hyperplastic endometritis may be seen following completion of an estrual cycle at any age but is most often observed in dogs over 6 years old. Virgins as well as parous females are afflicted with the disease, the most common cause of sterility in the bitch. The course may be chronic and intermittent or may end suddenly and unexpectedly in death. The clinical features include malaise, fever, prolongation or shortening of the estrual cycle with increase or decrease in the physical evidences of estrus, vulvar hypertrophy with swelling, hardening and secondary traumatic changes, gray-yellow to red-brown, usually putrid vaginal discharge, abdominal distention due to the enlargement of the uterus, simulating pregnancy, mammary tumors and sometimes inguinal hernia, attacks of lumbago, straddling gait of the hindlegs, dry, inelastic skin, sparse, dry, brittle hair, and varying degrees of alopecia with pigmentation especially on the dorsum, the sacrum, the flanks, the abdomen, the perineum and the pudendum. In the ovaries the following abnormalities were observed: cystic follicles, either solitary or multiple, tumors ranging from fibroma to epithelioma in 5 of the 24 cases, corpora lutea fairly fresh in early cases and variously regressed in chronic cases, and all stages of fol-

licle growth and regression. In the early phases, the uterus was enlarged, hyperemic, softened, distended and plicated, the cervix was small, and the ovaries contained fresh corpora lutea. In the later phases varying fibrosis, endometrial and serosal cysts, diverticula, solitary or multiple tumors and thickened cystic endometrium were present. Histologically, the endometrium displayed extreme glandular hyperplasia with edema and hyperemia, followed by retrogressive changes including necrosis, cystic change, abscess formation and fibrosis. Projections of hyperplastic endometrium extended into the myometrium, which was involved by hyperemia, edema and fibrosis, which also affected the cervix.

DeVita divided the cases of hyperplastic endometritis into two varieties. The first included those cases in which a metestrual type of pyometria or uterine abscess occurred and those in which this condition (because of the presence or reestablishment of cervical drainage) went into retrogression and some regression, to become activated again by completion of another cycle. Illustrative of the first point was a cocker spaniel bitch, 13 years old, which over a period of three years had several attacks of pyometra following completion of an estrual cycle or occurring at a time when an estrual cycle might have been completed. Bilateral oophorectomy and partial right uterine cornuectomy showed a cystic condition of both ovaries, which contained corpora lutea, and cystic glandular hyperplasia of the endometrium. At autopsy, three weeks later, the hyperplastic endometrial changes in the remnant of the right uterine horn had greatly regressed. Emphasizing the second point was the case of an old poodle with reactivated endometritis, many breeding failures, frigidity and shortening of the interestrual interval. The uterus showed fibrosis, extensive cystic change, edema, hyperemia and focal abscesses. The vagina was fibrotic and lined by papillary stratified squamous epithelium. The second variety of cases included those in which the hyperplastic endometritis was associated with ovarian tumor. These were typified in the case of a cocker spaniel bitch, 6 years old, which was mated in estrus to one dog and fourteen days later to another but did not whelp to either. She again accepted a male fifty-nine days after the first breeding. She was in a constant state of nymphomania from this time until one hundred and fourteen days later when panhysterectomy revealed a chronic fibrotic hyperplastic condition of the uterus and ovarian tumors.

In discussing the etiologic factors of hyperplastic endometritis in the dog, DeVita indicated that estrogen-producing ovarian cysts and tumors appear to be a probable source of the inciting

8 Mulligan<sup>1</sup>, Baldoni<sup>2</sup>, Krause<sup>3</sup>, Zuckerman and McKeown<sup>4</sup>, Goodpasture<sup>5</sup>, Hobday<sup>6</sup>, Moore<sup>7</sup>.

9 Wolfe, J. M., Cleveland, R., and Campbell, M. *Ztschr f Zellforsch u mikr Anat* **17** 420, 1933.

10 DeVita, J. *J Am Vet M A* **95** 50, 1939.

tactor when associated with some corpus luteum activity. This would also serve to explain the uterine changes in the dog reported on by Wolfe, Cleveland and Campbell.<sup>9</sup> In view of the abnormal cellular composition of the anterior lobe of the pituitary gland of this animal, future studies on "hyperplastic endometritis" might profitably be concerned with a histologic analysis of all the endocrine glands and secondary sex glands as well as of the parenchymatous viscera.

The only other neoplasm of an endocrine gland causing hormonal effects was reported by Slye and Wells<sup>11</sup> as occurring in a female white pit bull terrier, 12½ years old, which during life exhibited evidences of hypoglycemia, with low values for blood sugar, and the ability of knowing when to ingest carbohydrate for the relief of symptoms. At autopsy this animal had an adenocarcinoma of the pancreas with metastases in the peripancreatic lymph nodes, a benign adenoma of the pancreas, a mixed tumor of the right posterior mamma, 2 subcutaneous lipomas of the abdominal wall and a sebaceous adenoma of the forefoot. Another case of pancreatic cancer in a male dog was described by Bru,<sup>12</sup> but the only symptom listed was dyspnea. The pancreas was thickened and dense and was sprinkled with firm, white-yellow nodules consisting of islet tissue in the form of irregular palely stained sheets of cells with a rich vascular supply. The structure of the metastases in the liver, the bronchial lymph nodes, the right auricle of the heart and the lungs was much like that of the primary tumor.

#### NEOPLASMS OF THE MALE GENITAL TRACT

Kunnemann<sup>13</sup> reviewed the published descriptions of four testicular tumors in dogs and added a report of 8 of his own. Siedamgrotsky<sup>14</sup> reported the case of a 17 year old male dog with medullary sarcoma of the testis and metastases in the retroperitoneal lymph nodes. Pauer<sup>14</sup> found a carcinoma in the retroperitoneal testis of a 9 year old dog. Calve<sup>14</sup> observed a dog with a primary carcinoma of the testis and a metastasis in the liver. In a dog over 10 years old Duschaneck<sup>14</sup> observed a large cell alveolar sarcoma in retroperitoneal testes bound to the lumbar vertebrae. Kunnemann described 8 dogs with neoplasms as follows: a pinscher, 9 years old, with a carcinoma of the left inguinal testis, a

setter, 11 years old, with a sarcoma of the right scrotal testis, a Dalmatian, about 1 year old, with a sarcoma of the left scrotal testis, a spitz, 10 years old, with a sarcoma of the right inguinal testis, a terrier with a fibrosarcoma of the right scrotal testis, a dachshund, 6 years old, with an interstitial cell tumor of the left scrotal testis, a pinscher, about 8 years old, with an interstitial cell tumor of the right testis, and an old poodle with an interstitial cell tumor of the right testis. Goodpasture<sup>5</sup> reported 4 cases of carcinoma of the canine testis. In each instance the tumor had a multicentric origin from the regressed epithelium of the tubules. In 1 case the tumor metastasized to a pelvic lymph node.

Ball<sup>15</sup> recorded 2 cases of interstitial cell tumor of the testis, in both the seminiferous tubules were pushed aside and showed compression atrophy. Kunze<sup>16</sup> observed 13 dogs, 9 to 15 years old, with solitary or multiple, unilateral or bilateral testicular interstitial cell tumors, which varied from the size of a pepper grain to that of a small hazelnut. This author found that such new growths occurred often in the atrophic testes of old dogs, always remained within the confines of the tunica albuginea, caused no testicular enlargement and were well defined yellow-white nodules in their early development but in later stages showed hemorrhagic necrosis and encapsulation by connective tissue. A rich network of capillaries and little connective tissue were present within the tumors, the lipid content of the cells of which could be strikingly and characteristically demonstrated with the sudan IV stain. In a series of 15 old dogs, most of them over 10 years old, Bouffannais<sup>17</sup> observed 2 grossly visible testicular tumors, a seminoma and an interstitial cell tumor. Chambers<sup>18</sup> saw a fox terrier, 8 years old, with a sarcoma of the testicle.

Pallaske<sup>19</sup> studied the organs of 107 male dogs at autopsy. Of the 31 with so-called interstitial cell tumors of the testes, 9 were under 10 years of age and the rest were 10 to 16 years old. In 28 of the dogs with these testicular tumors he also found nodular hyperplasia of one or more of the following organs: liver, adrenal glands, spleen, pancreas and thyroid gland. This study emphasized the difficulty in a given case of interstitial cell growth of the testis of an old dog of

11 Slye, M., and Wells, H. G. *Arch Path* **19**:537, 1935

12 Bru, P. *Rev med-chir d mal du foie* **2** 40, 1927

13 Kunnemann, O. *Arch f wissensch u prakt Tierh (supp)* **36** 229, 1910

14 Cited by Kunnemann<sup>13</sup>

15 Ball, M. *Bull Assoc franç p l'étude du cancer* **11** 5, 1922

16 Kunze, A. *Virchows Arch f path Anat* **240** 144, 1923

17 Bouffannais. *Bull Assoc franç p l'étude du cancer* **18** 808, 1929

18 Chambers, F. *Vet Rec* **11** 709, 1931

19 Pallaske, G. *Virchows Arch f path Anat* **281** 856, 1931

determining whether the proliferated interstitial cells might constitute merely hyperplasia or true neoplasm and, once the neoplastic character of such a growth was established, whether adenoma or carcinoma might be the correct diagnosis. Among 1,111 dogs examined at autopsy Skoda<sup>20</sup> found 21 that had neoplasms of the testes, 14 of these dogs had abnormally and 7 normally located testes. On the other hand, Lehr<sup>20</sup> studied 969 surgically removed canine tumors, among which were 34 (3.5 per cent) testicular neoplasms, none of which was stated to have been in a cryptorchid animal.

Schlotthauer, McDonald and Bollman<sup>21</sup> studied 82 spontaneous testicular tumors in dogs at operation and necropsy. Tumors were present in 59 testes of the 48 dogs examined. The average age of the dogs with the 51 interstitial cell tumors was 12 years. In 7 testes 18 interstitial cell tumors of multicentric origin were discovered. In 8 cases the interstitial cell tumor was associated with seminoma in the same testis. The average age of the 25 dogs with seminoma was over 10 years. Eighteen of the seminomas were of multicentric origin, and 4 were encountered in undescended testes. Of the 3 dogs with bilateral seminoma, 2 had scrotal and 1 had inguinal testes. Eighteen seminomas were in situ, 17 were not associated with other tumors and 5 occurred alone with interstitial cell tumors. These authors observed 6 testicular adenocarcinomas of varying degrees of malignancy.

Zuckerman and McKeown<sup>4</sup> examined histologically the testes and prostates of 243 dogs of various breeds. They found 35 testicular tumors: 17 seminomas in dogs 8 to 15 years old, 15 adenocarcinomas in dogs 4 to 15 years old and 3 interstitial tumors in dogs 9 to 15 years old. Innes<sup>22</sup> collected 49 canine testicular tumors, 36 of which were surgically removed. The ages of the animals, accurately tabulated by Innes, ranged from 5 to 13 years in 46 of the 49 cases. In addition to these 49 testicular neoplasms, including 32 seminomas, 15 tubular adenomas or Sertoli cell tumors and 2 cancerous interstitial cell tumors, Innes also saw nodular hyperplasia of the interstitial cells of the testes in 12 dogs. He presented evidence that nodular hyperplasia of the interstitial cells is not truly neoplastic. In his follow-up of surgical cases and from autopsies he concluded that the seminoma in the dog is of low grade malignancy as compared with the corresponding tumor in man. Of 79 growths of the

testes of 393 dogs examined by Saloman,<sup>23</sup> 29 were seminomas, 41 were interstitial cell growths, 7 were mixed tumors and 2 were seminiferous adenomas, some of them multiple.

Although neoplasms of the testis comprise by far and away the greatest number of new growths observed in the canine male genital tract, several others of passing interest may also be mentioned briefly. Huebner<sup>24</sup> described the case of a male fox terrier, 2 years old, which had a bean-sized tumor of the dorsal surface of the penis and bilaterally enlarged inguinal lymph nodes. The dog was killed twenty-six days after the tumor had been extirpated, since it was blind. Autopsy disclosed metastases of small round cell alveolar sarcoma in the inguinal lymph nodes, in the right submaxillary lymph nodes and in the anterior chambers, irises and vitreous bodies of both eyes. These lesions were metastases from the primary sarcoma of the penis. Houdemer and Bablet<sup>25</sup> reported the case of a male mongrel dog, 18 months old, from which were surgically resected 2 nodules on the end of the penile sheath and abundant vegetations encircling the base of the penis and distending its sheath. The enlarged inguinal lymph nodes on the left side became infected and spontaneously obliterated, and those on the right side were resected twenty-four days later. The histologic diagnosis of the penile and lymph node lesions was lymphoblastoma. Little<sup>26</sup> saw a calcified squamous cell carcinoma of the penis of an old mongrel. Chambers<sup>18</sup> described a sheep dog, 10 years old, with a carcinoma of the prepuce.

Boucek<sup>27</sup> observed a dog with a fist-sized adenocarcinoma of the prostate and metastases in the pelvic and mesenteric lymph nodes. Described by Morini<sup>28</sup> was a wolf hound, about 2 years old, with an adenocarcinoma of the prostate which had extended widely to the pelvis and the pelvic viscera and metastasized to the kidneys, the liver and the mediastinal lymph nodes. Since the prostate also showed areas of adenoma, cystadenoma, fibromuscular hyperplasia and focal chronic inflammation, the author discussed the possibility of the change of a prostate involved in benign hypertrophy into one with an adenocarcinomatous character. Mori<sup>29</sup> reported the case of a great Dane, 8 years old, with a fist-

<sup>23</sup> Saloman, cited by Innes<sup>22</sup>

<sup>24</sup> Huebner, Berl tierarztl Wchnschr **38** 135, 1922

<sup>25</sup> Houdemer and Bablet, Bull Soc path exot **20** 344, 1927

<sup>26</sup> Little, G. W., J Am Vet M A **71** 171, 1927

<sup>27</sup> Boucek, Z., Arch f wissenschaft u prakt Tierh **32** 585, 1906

<sup>28</sup> Morini, E., Boll d Soc Eustachiana **29** 185, 1931

<sup>29</sup> Cited by Morini<sup>28</sup>

<sup>20</sup> Cited by Krause<sup>3</sup>

<sup>21</sup> Schlotthauer, C. F., McDonald, J. R., and Bollman, J. L., J Urol **40** 539, 1938

<sup>22</sup> Innes, J. R. M., J Path & Bact **54** 485, 1942

sized adenocarcinoma of the prostate and metastases in the testes, lymph nodes, the spleen and the lungs. Recorded by Boudet<sup>29</sup> was the case of a dog with a carcinoma of the prostate and a metastasis in the right cerebral hemisphere. Rudduck and Willis<sup>30</sup> described an Alsatian, 9 years old, with an adenocarcinoma of the prostate and metastases in lymph nodes, the lungs and a kidney.

#### NEOPLASMS OF THE FEMALE GENITAL TRACT

Exclusive of mammary tumors, the neoplasms of canine female genitalia recorded in the literature are relatively few. With respect to the gonads, the ovarian neoplasms reported are far behind testicular new growths. Bruckmuller<sup>31</sup> mentioned a carcinoma of a canine uterus with nodular extension into the uterine wall and an oviduct. In female dogs Boucek<sup>27</sup> saw a pedunculated fibromatous vaginal polyp, an ulcerated squamous cell carcinoma of the vagina and a walnut-sized leiomyoma of a gravid uterus. Johne<sup>32</sup> listed 1 uterine and 2 vaginal leiomyomas. Leisering<sup>32</sup> described a bitch with multiple submucous fibroleiomyoma of the vagina. In a 7 year old female brach hound Roquet<sup>33</sup> observed a pedunculated vaginal fibroma which hindered micturition by reason of its attachment in front of the urethral meatus. Belkin's<sup>34</sup> case 238 was that of a female Irish setter, 6 years old, with a submucous fibroleiomyoma on the posterior vaginal wall. Little<sup>26</sup> observed multiple neurosarcoma in the vagina of an English setter.

Coquot and Nenckoff<sup>35</sup> culled 18 ovarian neoplasms from 31 female dogs with abdominal tumors. Eight were ovarian and extraovarian cysts, 5 were cysts and adenomas, 3 were carcinomas, 1 was a lymphadenoma and 1 was a fibroma. The symptoms accompanying these ovarian tumors were increase in the size of the abdomen, panting, interference with locomotion and weakness and infrequency or absence of the estrual periods. The abdominal examination revealed a palpable, mobile solid mass which within limits could be grasped through the abdominal wall. The ovarian tumor was usually rounded, nodular, displaceable in the abdominal cavity by the thrust of the fingers, and was rendered immobile by distention

of the edematous ligament attaching it to the lumbar gutter. Depicted in this paper were a 9 year old shepherd bitch with a 5,300 Gm fibroma of the left ovary and an 8 year old hunting dog with cysts and adenomas of the right ovary weighing 6,600 Gm. Huggins and Moulder<sup>36</sup> mentioned a dog with carcinoma of the uterine cornua and 3 dogs with vaginal leiomyoma.

#### NEOPLASMS OF OTHER ENDOCRINE GLANDS

Not available for perusal was a paper on hypophysial tumors by Luksch<sup>37</sup> (1923). Other neoplasms occurring in the canine pituitary gland (aside from solitary or multiple cysts, which are rather common in dogs according to Stockard<sup>38</sup> and in my experience) were 2 adenomas of the anterior lobe, 1 recorded by Goodpasture<sup>5</sup> and the other by Joest, previously quoted<sup>39</sup>. White<sup>40</sup> described a male fox terrier, about 4 years old, which showed irritability, lethargy, polyuria, polydipsia and obesity six months before death. Autopsy disclosed small testes, a small prostate, a large thymus and a suprasellar craniopharyngioma which was about four times the size of a normal pituitary gland and invaded the mid-brain, the third ventricle, the crura cerebri and the optic thalami. No trace of a pituitary gland was discovered. White also mentioned 3 other tumors of the canine pituitary gland, including an adenocarcinoma (Joest), a chromophobe adenoma with the syndrome of adiposogenital dystrophy (Hare) and an adenoma (Belmonte).

Blair<sup>41</sup> reported the case of a Boston bull terrier, about 12 years old, which suffered from obesity, asthma, chronic cough and dyspnea. At autopsy a globular mass was adherent to the superior borders of the cardiac auricles, was attached quite firmly to the trachea and the esophagus and surrounded the pulmonary veins. The heart was greatly hypertrophied, the ventricles were dilated, and the atrioventricular valves were marked by vegetation and imperfect closure. Although the diagnosis of "round cell sarcoma in the heart" was made, the obvious conclusion is that this neoplasm was a lymphosarcoma of the thymus. A thymoma reported by Joest has been mentioned before<sup>39</sup>. Goodpasture<sup>5</sup> described a "reticular" tumor of the thymus.

30 Rudduck, H. B., and Willis, R. A. *Am J Cancer* **33** 205, 1938.

31 Bruckmuller, cited by Casper, M. *Ergebn d allg Path u path Anat* **3** (pt 2) 754, 1896.

32 Cited by Casper, M. *Ergebn d allg Path u path Anat* **11**(pt 2) 1068, 1907.

33 Roquet, M. M. *J de méd vet et de zootech* **59** 713, 1908.

34 Belkin, G. *Berl tierarztl Wchnschr* **41** 829, 1925.

35 Coquot, A., and Nenckoff, G. *Rec de med vet* **106** 129, 1930.

36 Huggins, C., and Moulder, P. V. *J Exper Med* **80** 441, 1944.

37 Luksch, cited by Winkler, K. *Ergebn d Biol* **5** 692, 1929.

38 Stockard, C. R., and others. *The Genetic and Endocrine Basis for Differences in Form and Behavior*, American Anatomical Memoir 19, Philadelphia, Wistar Institute of Anatomy and Biology, 1941, p. 454.

39 Mulligan, R. M. *Arch Path* **38** 115, 1944.

40 White, E. G. *J Path & Bact* **47** 323, 1938.

41 Blair, W. R. *J Am Vet M A* **49** 520, 1916.



To my knowledge no one has ever recorded an instance of a solid neoplasm of the parathyroid glands of a dog. On the other hand, the thyroid gland is frequently the site of nodular hyperplasia<sup>42</sup> as well as of true neoplasm. Ewald<sup>43</sup> reviewed 75 cases of cancerous canine goiter from the literature and added 5 new cases of his own. In 63 of the 80 cases the neoplasm was a carcinoma, in 6 a sarcoma and in 7 a mixed tumor (carcinoma and sarcoma coexisting). In 4 it was not classified as to histologic type. The cervical veins were invaded in 6 cases, and metastases involved the cervical lymph nodes in 3 cases. The other organs with metastases and their frequency were as follows: lungs, 33; spleen, 9; kidneys, 6; mediastinal lymph nodes, 5; heart, 4; liver, 4; testes, 2; and skin, adrenal glands, breast, and intestine, 1 each. Reports of other instances of cancer of the thyroid gland have been abstracted in previous papers.<sup>44</sup> Another case was described by Cremona<sup>45</sup>, a male pointer, about 7 years old, was observed with a polymorphous sarcoma of the thyroid gland and metastases in the lungs, the left kidney and the bronchial, mediastinal and left lumbar lymph nodes. Rudduck and Willis<sup>30</sup> saw a male sheep dog, 10 years old, with a mixed adenocarcinomatous and ossifying neoplasm of the thyroid gland and ossifying metastases in the lungs.

Neoplasms of the pancreatic islets<sup>46</sup> have already been mentioned. Aside from the nodular hyperplasia of the adrenal cortex discussed before,<sup>42</sup> no neoplasms of this structure have been found recorded. The cancer of the medulla of the adrenal gland described by Goodpasture<sup>5</sup> was the only true neoplasm of this organ discovered in the literature. This tumor invaded the adrenal veins and the inferior vena cava and metastasized to the liver.

#### INFECTIOUS OR VENEREAL SARCOMA

An introduction to this phase of canine oncology could not more suitably be obtained than by summarizing the experience of Beebe and Ewing<sup>47</sup> with infectious sarcoma of dogs and their analysis of the work of Sticker with respect to this disease. Sticker secured specimens of infectious tumors of the penis and the vagina studied by Wehr, Geissler, Smith and Washbourn, Sanfelice and Bashford and submitted

them with his own specimens to a large number of German pathologists, all of whom agreed on the diagnosis of round cell sarcoma. Supported by this diagnosis of recognized authorities on the structure of tumors, Sticker compared the general features of the disease with cancer in man and showed on these grounds that the growth must be accepted as a true cancer. The following statements constituted his proof:

1 Clinically, the other infectious venereal diseases of dogs resembling lymphosarcoma always cause prolonged inflammation and multiple nodules (merely enlarged lymph follicles), while inoculation of the vagina with the true tumor produces little, if any, inflammation, which soon subsides and is followed by a solitary tumor, or at best two to three tumors, arising in the subepithelial tissue.

2 Histologically, the tumor is entirely different from any known infectious granuloma, is not accompanied by any inflammatory reaction in the neighboring tissues and produces metastases, which develop from cells carried by the blood stream, without participation of the tissue cells.

3 Experimentally, living cells must be transferred in order to secure a growth, while tumor emulsion that has been finely comminuted or strained through a porcelain or paper filter is innocuous.

4 The spontaneous transference of the tumor is paralleled in the cases of "cancer à deux" and of carcinoma of the upper lip transferred from the lower lip in man.

Beebe and Ewing admitted that Sticker's argument presented a body of evidence strongly in favor of the opinion that infectious lymphosarcoma of dogs is a true tumor. Their own experience supported the views of Sticker, although they were unable then to distinguish satisfactorily between his follicular vaginitis and lymphosarcoma. Beebe and Ewing were forced to conclude that infectious lymphosarcoma of dogs is a true cancer on the basis of the work of others and of their own experiments with this disease. The incidence of venereal sarcoma varied with different authors. Auler and Wernicke<sup>48</sup> found 8 (1.4 per cent) tumors of this type among 585 canine tumors, Feldman<sup>49</sup> saw 7 (8.6 per cent) among 81 tumors in dogs, Stubbs and Furth<sup>50</sup> observed 5 (0.017 per cent) among 30,000 dogs observed in the animal clinic in Philadelphia, Vos<sup>51</sup> collected 5 (0.5 per cent) from among 1,100 Javanese dogs, and Kaalund-Jørgensen and

48 Auler, H., and Wernicke. *Ztschr. f. Krebsforsch.* **35** 1, 1931.

49 Feldman, W. H. *Neoplasms of Domesticated Animals*, Philadelphia, W. B. Saunders Company, 1932, pp. 343-356.

50 Stubbs, E. L., and Furth, J. *Am. J. Path.* **10** 275, 1934.

51 Vos, J. J. T. *Geneesk. tijdschr. v. Nederl.-Indie* **75** 263, 1935.

42 Mulligan, R. M. *Cancer Research* **4** 505, 1944.

43 Ewald, O. *Ztschr. f. Krebsforsch.* **15** 85, 1916.

44 Mulligan (footnotes 39 and 42).

45 Cremona, P. *Nuovo Ercolani* **26** 365 and 388, 1921.

46 Slye and Wells<sup>11</sup>. *Bru*<sup>12</sup>.

47 Beebe, S. P., and Ewing, J. A. *J. M. Research* **15** 209, 1906.



Thomsen<sup>52</sup> described 8 (0.6 per cent) among 1,400 dogs. Wong and K'ang<sup>53</sup> successfully treated 3 female dogs with infectious sarcoma of the vagina with radium. One bitch also had involvement of the inguinal lymph nodes, which subsided after radium therapy. A male dog with an infectious sarcoma of the penis was given several courses of radium treatment, following which stricture of the penile urethra developed with resultant uremia and death. This paper contained a good photomicrograph ( $\times 1,000$ ) of the tumor in their case 2.

Since infectious or venereal sarcoma of the dog is obtained spontaneously through coitus and since cohabitation of the dog is conditioned by receptivity of the bitch for the male, it is logical to assume that female dogs were first afflicted with this disease, although where it might have come from before the female dog acquired it is a matter of conjecture. Because the time of estrus, or the period of receptivity of the bitch for the male, is associated with the highest possible natural estrogenic stimulation of the canine female genital tract, notably the vagina, in consequence of the hormonal elaboration of the ripe ovarian follicles, the future investigation of venereal sarcoma in the dog might well be directed along the lines of hormonal research.

#### MAMMARY NEOPLASMS

Neoplasms of the mammary glands are probably as common as any occurring in dogs. Gibbes<sup>54</sup> saw an alveolar cell sarcoma in the mammary gland of a bitch. Kitt<sup>55</sup> described fibroma of the breast, seen frequently in dogs, as a sharply delimited nodular hard structure derived from the interstitial connective tissue and surrounding the mammary ducts and acini (fibroma pericanaliculare). He also mentioned myxofibroma of the canine breast. He stated that in the dog enchondroma is found chiefly in the breast, is often multiple and usually appears in association with mixed tumors. In the breasts of bitches he also observed osteoma and chondroosteoma. In most instances they were sharply circumscribed, round and nodular, but they also occurred as knotty flat bony bars.

Stockfleth-Bang<sup>52</sup> described a lipoma in the breast of a fat bitch and said that in the breast lipoma may reach a tremendous size. Lucet<sup>52</sup>

reported a 1,700 Gm myxoma of the right third breast of a 6 year old female sheep dog. Stenzel<sup>52</sup> described a 2,000 Gm mammary chondroma in a dog. In this tumor he found many areas in which embryonal cartilage tissue was in various stages of development. He also reported 2 cases of cystic fibrochondroma of the canine breast. Grischn<sup>52</sup> removed from the breast of a bitch a mobile hard osteochondroma the size of a hen's egg. Petit<sup>52</sup> observed 2 mammary tumors, a pure chondroma and an osteochondroma, in the same breast of a dog.

Freese<sup>56</sup> was the first to review rather extensively the early literature on canine mammary neoplasms. He mentioned some cases already cited by Casper<sup>52</sup> and many others. He also reported 7 cases of his own. In the early literature he found reports of several varieties of canine mammary tumors, including fibroma or fibroadenoma (McFadyean, Ball and Leblanc, Johne), myxoma (McFadyean), chondroma (Peuch, McFadyean, Frohner), fibromyxochondroma (Johne), sarcoma (Peuch, Molle, Johne, McFadyean), sarcoma with widespread metastases (Petropawlowsky), chondrosarcoma (Johne and Frohner), cystadenoma and adenocarcinoma (Johne) and carcinoma (Semmer, Johne, Casper, Frohner). Putz<sup>57</sup> saw a dog with multiple mammary carcinoma and metastases in the lungs and the left elbow joint. Petit<sup>57</sup> described an old bitch with a mammary carcinoma and metastases in the liver, the spleen, the lungs and other organs. Kitt<sup>57</sup> observed a female dachshund with a mammary cystocarcinoma and metastases in the lungs. Stenzel<sup>57</sup> found an adenocarcinoma of the type of embryonic mammary anlagen in the breast of a bitch.

Freese<sup>56</sup> studied both grossly and microscopically 7 canine mammary neoplasms which may be listed as follows: (1) a cystochondrofibrosarcoma of the last four breasts of a spitz 11 years old, (2) a cystadenosarcofibroma of the left penultimate breast of a mongrel spitz 16 years old, (3) an angiofibrosarcoma of the left hindmost breast and an osteochondrofibrosarcoma of the left penultimate breast of a dachshund 11 years old, (4) a cystadenofibrosarcoma of the left penultimate breast of an English greyhound 4 years old, (5) a myxofibrosarcoma of the right penultimate breast and a cystofibroadenoma of the right hindmost breast of a dachshund 13 years old, (6) a papillary cystadenoma of the left penultimate breast of a cart dog 5 years old, (7) a papillary cystadenoma of the right caudal breast of a St Bernard 13 years old. No

<sup>52</sup> Kaalund-Jørgensen, O., and Thomsen, A. S. *Ztschr f Krebsforsch* 45 385, 1937.

<sup>53</sup> Wong, A. I. H., and K'ang, H. J. *Chinese M J* 46 377, 1932.

<sup>54</sup> Gibbes, cited by Sutton, J. B. *J Anat & Physiol* 19 415, 1884-1885.

<sup>55</sup> Kitt, cited by Casper, M. *Ergebn d allg Path u path Anat* 3(pt 1) 692 1896.

<sup>56</sup> Freese, K. *Ztschr f Tiermed* 9 206, 1905.

<sup>57</sup> Cited by Freese<sup>56</sup>.

metastases were seen at autopsy in any of the dogs. In the first 5 tumors pericanalicular tissue was most prominent, in the last 2 glandular tissue was paramount. According to Freese,<sup>56</sup> there occurs in the breasts of dogs a group of tumors with stroma composed of mesenchymal tissue that was broken off in embryonic life and subsequently began to grow. This mesenchymal tissue is undifferentiated and is variously ready to produce cells of connective tissue origin. Even more certainly, the epithelial structures of these tumors are derived from ectodermal sources and lie together with the mesenchymal elements in a resting state until, later, they begin to grow. These tumors are sharply delimited from the adjacent tissue and may be recognized as foreign bodies. These encapsulated mammary tumors are without exception mixed tumors, formed from various types of connective tissue elements and epithelial structures. They are usually benign and can be treated by operation. This discussion by Freese of the variegated structure seen in his own cases of mammary neoplasm as well as in those recorded in the early literature emphasizes their extremely complex structure and helps to explain many cases recorded since 1905.

Ortschild<sup>58</sup> recorded 5 mammary cancers as follows: (1) a cystadenocarcinoma of the right hindmost breast with metastases in the regional lymph nodes of a nulliparous Skye terrier bitch 16 years old, (2) an adenocarcinoma of the "second left thoracic breast" with metastases to axillary, thoracic and inguinal lymph nodes of an old Skye terrier bitch, (3) a cystadenocarcinoma of the third right breast with metastases in the opposite breast and the second breasts of an old bitch, (4) an adenocarcinoma of the right hindmost breast of a Gordon setter bitch 8 years old, (5) a papillary carcinoma of the right hindmost breast with metastases in inguinal lymph nodes of a Gordon setter bitch 10 years old. Foreman<sup>59</sup> reported the case of an Irish terrier bitch 11 years old from whose hindmost breast a 1 pound (0.5 Kg) osteochondroma was extirpated. Seven months later autopsy disclosed metastatic osteochondroma in both the liver and the spleen. Goodpasture<sup>5</sup> saw 2 carcinomas of the female canine breast among 13 cancers culled from 50 old dogs.

Corsy and Thomas<sup>60</sup> found in a series of 50 canine mammary tumors, surgically removed, a liposarcoma occupying an entire breast. They

also discussed 9 cases of human liposarcoma recorded in the literature. Among over 100 tumors of the canine breast surgically removed at the Alfort Veterinary School, Petit and Peyron<sup>61</sup> found a mammary tumor in which co-existed a predominantly epithelial mixed tumor and a liposarcoma.

Little<sup>28</sup> tabulated 12 canine neoplasms, among which were 5 located in the mammae of bitches as follows: (1) an adenocarcinoma of the left most caudal mamma of a Boston bull 9 to 10 years old, (2) a low grade mammary adenocarcinoma in a poodle, (3) an adenocarcinoma involving the first two right breasts and the left first breast, with metastases in the lungs and the spleen of a Boston terrier 13½ years old, (4) a mammary carcinoma in a fox terrier, and (5) a papillary adenochondrocarcinoma of the breast of a spaniel. Chambers<sup>18</sup> observed 15 histologically confirmed cases of canine cancer, among which was a recurrent mammary fibrosarcoma in an 11 year old fox terrier bitch. Rudduck and Willis<sup>30</sup> saw a female cocker spaniel with a cystic papillary mammary carcinoma.

Baldoni<sup>2</sup> reviewed cases of tumor of the canine male breast reported by Vachetta, Petit and Cinotti. Vachetta's case was one of spindle cell sarcoma. Petit described a male dog with a sarcomatous carcinoma of the breast and sarcomatous, carcinomatous and sarcomatous metastases in the lymph nodes and the lungs. Cinotti recorded the case of a male pug, 13 years old, with a mixed tumor (cystadenochondroma) of the right first abdominal breast. In addition to these Frohner reported 1 and Murray<sup>2</sup> neoplasms of the canine male breast, which have been referred to in a previous review.<sup>30</sup>

The most common locations for mammary tumors in the dog are the posterior breasts. Of 114 mammary tumors studied and definitely located by Antoine, Liégeois and Verstraete,<sup>62</sup> 5 were situated in the first breasts, 18 in the second, 13 in the third, 36 in the fourth and 42 in the fifth. In 31 female dogs Huggins and Moulder<sup>36</sup> found 120 mammary growths of appreciable size, varying from solitary cysts to large solid tumors. These tumors were distributed in the breast as follows: first, 9, second, 18, third, 21, fourth, 36, and fifth, 36. The histologic types observed were sarcomatous, carcinomatous and papillary cystic. Metastases, consisting of intracystic papillary epithelial

<sup>58</sup> Ortschild, J. F. *Bull. Johns Hopkins Hosp.* **16** 186, 1905.

<sup>59</sup> Foreman, R. J. *Vet. J.* **69** 240, 1914.

<sup>60</sup> Corsy and Thomas. *Bull. Assoc. franç. p. l'étude du cancer* **16** 143, 1927.

<sup>61</sup> Petit and Peyron. *Bull. Assoc. franç. p. l'étude du cancer* **16** 510, 1927.

<sup>62</sup> Antoine, G., Liégeois, F., and Verstraete. *Bull. Acad. roy. de med. de Belgique* **14** 301, 1934.

tumors, were found in the lungs of 3 dogs and in the lungs and liver of a fourth

In the experience of DeVita,<sup>10</sup> the growth of most mammary tumors in female dogs apparently parallels the growth of the endometrium during metestrus, their growth beginning possibly during estrus. They seem to be activated into growth at this time and then go into a quiescent phase during which they may become sclerotic. At the next estrus, growth seems to be reestablished, and they either increase in size or undergo regressive change, which in some instances results in complete destruction of the tumor through sloughing. The irregular growth may extend over several cycles. In 10 of 11 instances of mammary adenomatosis in which he also examined the uterus, DeVita observed atypical endometrial hyperplasia, this supports the assumption that estrogenic stimulation might be responsible for both the mammary and the uterine changes.

In considering mammary carcinoma in dogs from the point of view of etiology Little<sup>26</sup> said

There is some evidence that a disturbance in the functional activity of the mammary glands, such as improper drainage of the milk flow resulting in mastitis, bears a causative relation to the late development of mammary carcinomas. This is frequently noticed in middle-aged or old virgin dogs, where a milk flow appears approximately 2 months after the oestral period—a phenomenon occurring in non-pregnant bitches which has never been explained. Sometimes this lactation persists for 6 weeks to 2 months.

In their experiments with commercial betanaphthylamine, with which they produced tumor of the bladder in female dogs, Hueper, Wiley and Wolfe<sup>63</sup> observed swollen and lactating mammary glands in many dogs on various occasions during the latter third of the experimental period of over two years. The posterior mammae were most often affected. Whether the carcinogen was directly or indirectly responsible for this change was not elucidated.

On the basis of these data and of those presented in two other papers<sup>44</sup> it may be said that mammary neoplasms are among the most common tumors in dogs, occur most frequently in dogs 6 years of age or older, are obviously heavily preponderant in female dogs although

they do occur in male dogs, may often be multiple, vary greatly in size and are most commonly located in the posterior breasts. The mixed tumor character of many of these growths is revealed in the varying combinations of cancerous and noncancerous changes in the cystic, solid and papillary cystic epithelial proliferations and in the growth of connective tissue, cartilage, bone, myxomatous tissue and even of adipose tissue. The types of tumors described include fibroma, fibroadenoma, osteoma, chondroma, osteochondroma, lipoma, myxoma, adenoma, cystadenoma, carcinoma, adenocarcinoma, cystadenocarcinoma, sarcoma, fibrosarcoma, chondrosarcoma, osteosarcoma, liposarcoma and myxosarcoma as well as varying combinations of these both cancerous and noncancerous neoplasms. Metastases of canine mammary cancer may be carcinomatous, sarcomatous or both. From the point of view of etiology two ideas have been advanced concerning the canine mammary neoplasms. The first is that they may be the result of congenital cell rests taking on abnormal growth characteristics. The second is that estrogenic stimulation of the mammary gland over several cycles may be responsible for their production. In the light of newer concepts of carcinogenesis it may appear that these two factors are but predisposing for one or more other factors responsible for actual neoplasia and even anaplasia.

#### SUMMARY

A review of the literature on canine oncology revealed several leads which might be followed in future investigation of neoplasms of the endocrine glands and of the genital tracts of both sexes. These included a syndrome of feminization in male dogs associated with testicular carcinoma, the relation between benign prostatic hypertrophy and castration, the coexistence of changes in the female genital tract with cysts and solid tumors of the ovary, the presence of hyperinsulinism with neoplasms of the pancreatic islets, the problem of the interpretation of proliferation of interstitial cells of the testes, the paucity of neoplasms reported for the pituitary, parathyroid, thymus and adrenal glands, the possible etiologic connection of hormones with venereal sarcoma, and the embryologic and hormonologic aspects of mammary tumors.

<sup>63</sup> Hueper, W. C., Wiley, F. H., and Wolfe, H. D. *J. Indust. Hyg. & Toxicol.* **20**: 46, 1938.

# A THEORY OF TRANSPOSITION OF THE ARTERIAL TRUNKS BASED ON THE PHYLOGENETIC AND ONTOGENETIC DEVELOPMENT OF THE HEART

MAJOR MAURICE LEV

MEDICAL CORPS, ARMY OF THE UNITED STATES

AND

OTTO SAPHIR, M.D.

CHICAGO

The nature of the abnormality in transposition of the large arteries has intrigued pathologists for many years. The various theories that have been propounded have been reviewed elsewhere.<sup>1</sup> We have been engaged in studying this problem since 1935. In 1937 we presented a possible explanation of the anomaly.<sup>1a</sup> Since then we have enlarged our experience and have somewhat altered our original theory. We wish to present here our present conception of this anomaly based on our present knowledge of the phylogenetic and ontogenetic development of the heart.

## THE ONTOGENETIC AND PHYLOGENETIC DEVELOPMENT OF THE HEART

Transposition of the large arteries cannot be understood without a knowledge of the evolution of the mammalian heart. This in turn is dependent on a knowledge of the evolution of the circulation of the vertebrates and the evolutionary progress of life as it emerged from sea water to fresh water and from fresh water to land. Already in the higher invertebrates (Annelida) a closed circulation is present. As the invertebrates changed their habitat from sea water to fresh water, the new environment led to the development of the vertebrates. Some of the vertebrates then migrated to land, while most others went back to the sea. The further evolutionary progress of the vertebrates was concerned with those that took up terrestrial life.

The outstanding characteristics of the terrestrial habitat are (1) the presence of a relatively tremendous amount of oxygen in the atmosphere, (2) the low viscosity of the air, permitting rapid

motion, (3) the marked fluctuations in the temperature and (4) the relative absence of water. The evolutionary progress of animal life on land is correlated with the increasing ability of vertebrates to utilize this environment and overcome its obstacles. Already in fresh water osmotic independence had been achieved. To survive on land further adaptations were necessary, such as changes in the metabolism of nitrogen and adjustments for conservation of water and utilization of oxygen and finally for control of heat. The development of the circulation, although correlated with all these adaptations, was mostly related to the increasing ability of the organism to utilize oxygen.

The change from a water to a land environment necessitated a change from the gill type of respiratory apparatus. With the great increase in available oxygen lungs were developed with a gradually increasing amount of respiratory epithelium. The increased absorption of oxygen and the increased liberation of carbon dioxide necessitated fundamental changes in the transport of these substances and in the giving of the one to, and the removal of the other from, the body cells. In other words correlated with the tremendous increase in the oxygen content of the environment and the resultant gradual evolutionary changes in the ability to take in this substance, there ensued a gradual concomitant adaptation in the efficiency of the circulation. This adaptation was made by (1) an increase in the efficiency of the pump, (2) an increase in the oxygen capacity of the circulating medium and (3) an increase in capacity for oxygen exchange between the blood and the tissues.

The increased efficiency of the pumping system was obtained by (1) a gradual increase in pressure (Redfield<sup>2</sup>), (2) an increase in rate (Redfield<sup>2</sup>) and (3) a gradual separation of arterial and venous blood. Our task, therefore, is to analyze the morphologic changes in the heart

From the Department of Pathology, Michael Reese Hospital.

This study was aided by a grant from the A. B. Kuppenheimer Fund. Research in this department is in part supported by the Michael Reese Research Foundation.

1 (a) Lev, M., and Saphir, O. J. Tech. Methods 17: 126, 1937. (b) Harris, J. S., and Farber, S. Arch. Path. 28: 427, 1939.

2 Redfield, A. C. Quart. Rev. Biol. 8: 31, 1933.

producing this increased efficiency of the pumping system. Because of our problem of transposition a more detailed study must be made of the adaptational changes in the bulbus in the evolutionary series.

Beginning with the elasmobranch, the heart is more or less an undivided tubular heart consisting of a sinus venosus, a single auricle, a single ventricle and a bulbus leading into a common arterial trunk<sup>3</sup>. Blood from the arterial trunk is fed to the various gill arches for oxygenation and is then sent through the systemic circulation to the body cells. Un氧ogenated blood is then returned to the heart by the venous circulation (fig. 1). In this type of uncomplicated circulation the bulbus, which is the lowest type of bulbus seen in the vertebrates, may be considered to have the following functions: (1) to prevent reflux of the blood into the ventricle, (2) to aid in propulsion of the blood and (3) to safeguard the fragile capillaries of the gills from excessive pressures (Keith<sup>4</sup>). In this species it is a comparatively long straight muscular tube in which the cavity is divided by four longitudinal ridges (anterior, posterior, right and left) extending throughout its length (fig. 2). These ridges are jammed together during cardiac contraction, thus preventing regurgitation into the ventricle (Robertson<sup>5</sup>). In the ganoid fish a similar undivided tubular heart and straight bulbus is present. However, instead of ridges in the bulbus, there are eight long rows of pocket valves, four of them (anterior, posterior, right and left) being more prominent than the others.

Thus in these early vertebrate forms both the internal respiratory (systemic) and the external respiratory (gill) circulation are controlled by one pump. This conforms to the relatively low amount of oxygen and carbon dioxide exchange in these forms. The bulbus here partakes in the propulsion of blood in addition to carrying on its valvular and gill-protecting functions.

A radical change in the circulation occurred in the dipnoan fish. This fish achieved air breathing in part and developed lungs. The increase in respiratory epithelium and the concomitant increase in oxygen and carbon dioxide exchange were accompanied by an increase in the size of the internal respiratory (pulmonary) circulation. This in turn was accompanied by a division of the pump system. The first stage of this is seen in the formation of the multi-axial auriculoven-

triculobulbar loop and the bayonet-shaped bulbus. By this method a relatively larger pumping power was obtained to cope with the necessity of increased pulmonary flow. This formation was correlated with the beginning formation of septums in the auricles and the ventricles, the formation of a bayonet-shaped bulbus containing a spiral valve and a twisting of the circulation of 180 degrees. This twist made possible an interchange of blood between the as yet only slightly divided circulations. The possible hydrodynamic explanations for these changes have been offered by Spitzer<sup>6</sup> and will be discussed later.

The changes in the bulbus of the dipnoan fish merit further attention. These have been described by Robertson<sup>5</sup> (fig. 2). The bulbus of the dipnoan fish, in contrast to the straight muscular tubular bulbus of the ganoid fish, is kinked in bayonet form, with a thinning out of the musculature of the ventral and lateral walls of the middle segment and the disappearance of the valves on these walls. Also in other segments of the bulbus the valvular structures have become smaller except at the distal end of the distal part of the bulbus, where they are still large and apparently functioning. In addition, a spiral septum with a twist of 270 degrees has been formed, going from right cushion 1 distally to left cushion A proximally. This extends from the middle of the distal to the proximal end of the proximal part of the bulbus. In the lepidosiren this ridge extends throughout the whole course of the bulbus, from the distal to the proximal end. In addition there is now a left ridge 3 in the distal bulbus. In both the ceratodus and the lepidosiren the proximal segment of the bulbus has a thick muscular coat, while the musculature of the distal and middle segments is relatively thinned.

These facts show that already in the dipnoan fish the muscular power of the bulbus was reduced, the muscle power being vested mostly in the ventricle, and the valve function in the bulbus had begun to be concentrated in the bulbus-truncus junction. In addition the bulbus was subjected to the process of septation just as were the other chambers of the heart. Thus the heart of the dipnoan fish was adapted to a greater intake of oxygen by an increase in pumping power and a beginning separation of the circulations. In the Amphibia the circulation was not much altered from that in the Dipnoi. The auricles are more completely separated, but there is still a common ventricle and the bulbus possesses the characteristics of the dipnoan bulbus.

<sup>3</sup> Already in the lowest crinates the heart tube is not straight but is kinked on itself in one plane. This kink differs in complexity from the multi-axial loop of the dipnoan and higher vertebrates.

<sup>4</sup> Keith, A. *Lancet* 2 1267, 1924.

<sup>5</sup> Robertson, J. I. *J. Path. & Bact.* 18 191, 1913.

<sup>6</sup> Spitzer, A. *Virchows Arch. f. path. Anat.* 243. 81, 1923.

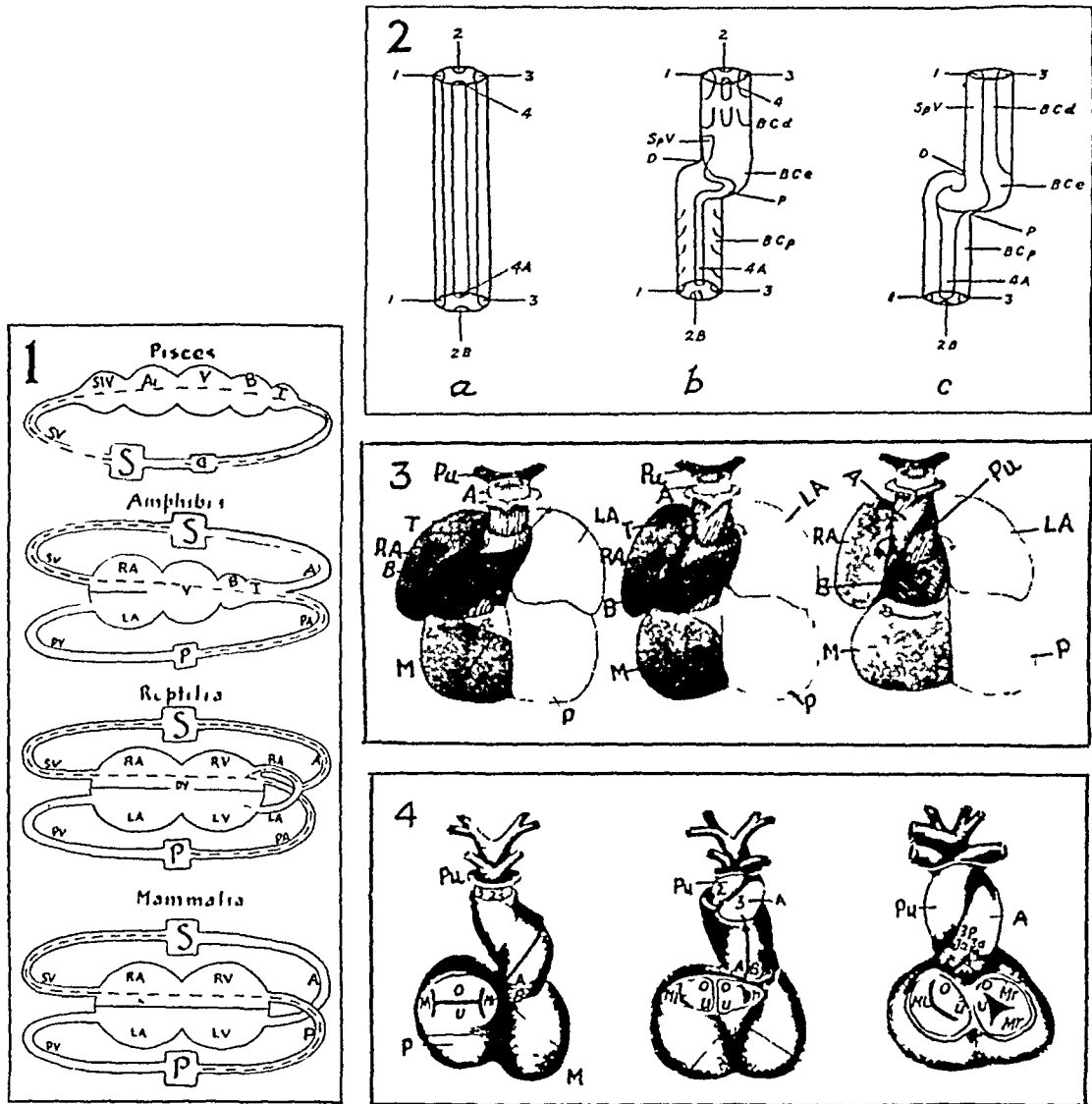


Fig 1—Diagrammatic sketches of the circulation in the phyletic tree. Pisces. The heart is a single tubular structure, consisting of a sinus venosus, an auricle, a ventricle and a bulbus (*SV*, *A*, *V* and *B* in the diagram). Un-aerated blood is pumped from the heart through the truncus (*T*) to the gills (*G*). The now aerated blood goes to the systemic cells (*S*). From there the venous blood is returned (*sv*) to the heart.

Amphibia. The auricular septum is now developed as well as the spiral valve (or partial septum) of the bulbus. The aortic portion of the bulbus is now decreased in size. These changes coincide with the appearance of the pulmonary vein and artery (*PV* and *PA*). Aerated blood in the left auricle (*LA*) and un-aerated blood in the right auricle (*RA*) enter the common ventricle (*V*), where considerable mixing occurs. Mixed blood is then propelled from the ventricle into the bulbus (*B*), and from the bulbus it is propelled spirally through the truncus (*T*) into the systemic and pulmonary circuits (*A* and *PA*) from which it is returned (*sv* and *pv*) to the heart. *P* indicates the lungs.

Reptilia. A complete auricular and a partial ventricular septum has now formed (*DV* indicates the defect of the interventricular septum). The bulbus has almost completely disappeared. A right ventricular (left) and a left ventricular (right) aorta (*RA* and *LA*) are present. Un-aerated blood is thus pumped from the right ventricle into the right ventricular (left) aorta and the pulmonary artery. Blood from the left ventricle is pumped into the left ventricular (right) aorta.

Mammalia. The auricular and ventricular septums are completely formed. The bulbus is completely absorbed. There is now complete separation of the systemic and the pulmonary circulation in the heart.

Fig 2—Diagrams of the bulbi of (a) *Elasmobranchii*, (b) *Ceratodus* and (c) *Lepidosiren paradoxa* (from Robertson<sup>5</sup>). Note the formation of the spiral fold from right-sided ridge 1 in the distal portion, and the ventral ridge 4*A* in the proximal portion. 1, right bulbar ridge 2 distal and 2*B*, proximal, part of the dorsal bulbar ridge 3, left bulbar ridge 4, distal, and 4*A*, proximal, part of ventral bulbar ridge *BCd*, distal segment of the bulbus *BCp*, proximal segment of the bulbus *BCe*, transverse segment of the bulbus *D*, distal constriction of the bulbus *P*, proximal constriction of bulbus *SpV*, spiral valve.

Fig 3—Second phase of the development of the heart—the absorption of the bulbus (from Pernkopf and Wirtinger<sup>7</sup>). *LA*, left auricle *RA*, right auricle *P*, proampule *M*, meta-ampule *B*, bulbus *T*, truncus *Pu*, pulmonary artery *A*, aorta.

Fig 4—Second phase of the development of the heart—changes in the auricular canal (from Pernkopf and Wirtinger<sup>7</sup>). *O*, *U*, *ML*, *MR*, endocardial cushions *A*, *B*, proximal bulbar cushions 1, 2, 3, 4, distal bulbar cushions *Pu*, pulmonary artery *A*, aorta *M*, meta-ampule *P*, proampule.

In the reptile further modification of the circulation occurred, producing a more complete separation of the pulmonary and systemic circulations and an increase in the efficiency of each. The auricular septum is now completed and only a defect remains in the ventricular septum (except in the crocodile, in which it is complete). The bulbus now underwent still more marked changes from that present in the Amphibia, changes directed toward its almost complete absorption into the ventricles, with the transmission of its functions to the ventricles and the truncus. Thus in *Lacerta* during embryologic development the bulbus becomes shortened and straightened with the disappearance of the distal and middle segments. The proximal portion is absorbed into the ventricles, all except a comparatively narrow rim of muscle at the base of the great arterial trunks (Robertson<sup>5</sup>). The bases of the great vessels are now guarded by semilunar valves. Thus, in addition to a diminution in the muscular power of the bulbus which had already occurred in the Dipnoi, the actual absorption of the bulbus and its elimination as a cardiac chamber must have been begun in the primeval reptiles and has proceeded far in present day reptiles. The functions of this chamber were taken over by other parts of the heart tube. Its valve function, to prevent regurgitation of blood into the ventricles, was taken over by the semilunar valves. Most of its musculature disappeared as contractile power was concentrated in the ventricles. And, according to Keith,<sup>4</sup> the portion of the bulbus which remained as the infundibulum of the right ventricle retained its function of shock absorber to the pulmonary circulation.

Pernkopf and Wirtinger<sup>7</sup> have analyzed the process of the absorption of the bulbus as it occurs in the reptiles. It may be subdivided into two components. First to appear is torsion at the ostium bulbotruncare in a counter-clockwise direction (if one is looking bulbusward from the ventricles). Then this becomes augmented in higher reptiles by back torsion (clockwise if one is looking bulbusward from the ventricle) at the ventriculobulbar ostium. By these processes the twist of the bulbar ridges is almost completely eliminated and the bulbus is absorbed into the ventricles. The first process (torsion at the ostium bulbotruncare) occurs maximally in lower reptiles—*Lacerta* and land turtles, in which it is greater than in mammals but in which ventral deviation and back torsion of the proximal bulbus are minimal. In the sea turtles a moderate amount of each is present. In the crocodiles

there is less torsion at the distal bulbar ostium with more back torsion at the proximal bulbar ostium. Thus apparently there is an inverse relation between torsion at the distal bulbar ostium and back torsion and ventral deviation at the proximal ostium. Both of these processes result in the elimination of the twist of the bulbar ridges, telescoping of the bulbus and its absorption into the ventricles. In addition back torsion and ventral deviation of the bulbus facilitate the completion of the ventricular septum. Thus the reptilian embryo goes through an additional process in the development of the heart. In addition to the formation of the auriculo-ventriculobulbar loop and the bulbar bayonet, with greater perfection of septation, it has now gone a step further in the absorption of the bulbus.

The completion of the task of separating the circulations with the development of greater efficiency in each occurred in the mammal. Here the embryo in its development, in addition to recapitulating the embryonic stages of its phylogenetic ancestors (Needham<sup>8</sup>), went a step further in the absorption of the bulbus, and completed the process of septation and the separation between the systemic and pulmonary circulations. Pernkopf and Wirtinger<sup>7</sup> have presented a very complete picture of this process.

The mammalian heart in its early development is an undivided tubular structure reminiscent of the elasmobranch and the ganoid fish, consisting of a sinus venosus, an auricle, a ventricle and a bulbus. The movements of the heart from its straight tubular form to its definitive condition proceeds in two phases. In the first phase (1 to 7 mm embryo) the auriculoventriculobulbar loop and bulbar bayonet are formed. During these formations a torsion of 90 degree (clockwise, looking ventricleward from the auricle) occurs at the auriculoventricular region, and an opposite torsion of 90 degrees (counterclockwise, looking bulbusward from the ventricle) occurs at the bulboventricular region. These bring the mesocardial (dorsal) portion of the ventriculobulbar orifice to the left, and the mesocardial (dorsal) portion of the auricular canal to the right. At 4 to 5 mm of fetal length the anlage of septums begins to be laid down in all portions of the heart tube (septum primum) as are the counter ridges of the auricle, the endocardial cushions, the main and the counter ridges of the ventricle, the proximal bulbar cushions, A and B, and the distal bulbar cushions, 1, 2, 3 and 4. These ridges are laid down on heterogonial (pertaining to the opposite anlage) parts of the heart tube sub-

<sup>7</sup> Pernkopf, E., and Wirtinger, W. *Ztschr f Anat u Entwicklungsgesch* 100 563, 1933

<sup>8</sup> Needham, J. *Biol Rev* 5 142, 1930



sequent to the beginning and simultaneous with the later course of the development of the first phase of the movements of the heart. When first seen the auricular ridges have a negative torsion of 90 degrees, the ventricular ridges practically no torsion and the bulbar ridges a positive torsion of 270 degrees. It is thus evident that the formation of the multi-axial auriculo-ventriculobulbar loop and bulbar bayonet results in the laying down of a "heterogonial" septal anlage with resultant twisting of the as yet incompletely divided circulations by 180 degrees. By this method the future exchange of blood between the two circulations is effected. This process, which we have now outlined more fully, has already occurred, as mentioned, in the embryologic development of the Dipnoi and is present in all higher animals. It is correlated with the necessities arising from the change to air breathing and the appearance of lungs in the phylogenetic series.

The mammalian embryo proceeds now to its second phase of development (figs 3' and 4). This phase concerns itself mainly with the absorption of the bulbus (fig 3), a process that occurs only in amniotes (reptiles, birds and mammals) but, as mentioned is incomplete in reptiles.<sup>9</sup> In mammals the absorption of the bulbus is effected by the two processes which we have outlined as they are seen in reptiles but in different degrees. Thus a torsion of 150 degrees (counterclockwise, looking truncusward from the bulbus) occurs at the ostium bulbotruncare, and back torsion of 45 degrees (clockwise, looking bulbusward from the ventricle) (first 90 degrees then reduced to 45 degrees) at the ostium ventriculobulbare. The latter is accompanied by a deviation directed ventrally and to the left of the bulbus. These movements are further correlated with a shift of the auricular canal to the right (fig 4) with expansion of the tricuspid orifice and of the dorsal wall of the meta-ampule (distal portion of the primitive ventricle), shrinkage of that part of the proampule (proximal portion of the primitive ventricle) which borders on the cranial wall of the auricular canal, and shrinkage of the mesocardial wall of the proximal bulbus. In this way the bulbus is absorbed into the ventricles, its mesocardial (aortic) portion disappearing and its ventral portion assuming the role of conus of the right ventricle. The functions of the bulbus are now taken over by other parts of the heart. Its

contractile function is taken over by the ventricles. Its valvular function of preventing back-flow of blood is taken over by the semilunar valves, and its function of acting as a shock absorber for the pulmonary circulation is possibly taken over by the conus of the right ventricle (Keith<sup>4</sup>). The two circulations have now been completely separated by a completed ventricular as well as an auricular septum.

Thus the mammalian embryo has gone a step further than its reptilian ancestors. It has now reached what at present is the highest form in the evolutionary development of the heart—a complete separation of the circulations for internal and external respiration, correlated with the gradually increased ability to take in oxygen. (This is present also in the birds.) This ability is correlated with a gradual increase in blood pressure and rate in the vertebrate series culminating in the mammal (Redfield<sup>2</sup>), making possible the better transport of oxygen.

To understand more fully the transition from the reptilian to the mammalian stage of circulatory development (which is necessary for adequate interpretation of congenitally malformed hearts), a comparison of the bulbus and the truncus of the two species must be made. In the truncus, in both the crocodile and the mammal the septum aortopulmonale develops. Because of the presence of a fifth branchial arch in the reptile, this septum develops from the spurs between arches 4 and 5 in the reptile, while in the mammal it originates from the spurs between arches 4 and 6. In both species the fusion of these spurs and their caudal extension divide the truncus into a dorsal pulmonary portion and a ventral systemic portion. However, there is a difference in the relative size of the pulmonary portion as seen by the attachment of the septum aortopulmonale to the distal bulbar cushions, 1 and 3. In the reptile the attachment is more toward the mesocardial end of these cushions while in the mammal it is more toward the center of these cushions. In the reptile in addition to the septum aortopulmonale a septum aorticum is formed. This originates from the spur between left arches 3 and 4, extends downward throughout the truncus, with its counterextension sinking into the right portion of the septum aortopulmonale. This thus divides the anterior portion of the truncus into a small left portion consisting of the fourth left arch, and a larger right portion leading into both carotid arteries and the right fourth arch. The left portion connects with the right ventricular aorta, while the right portion connects with the left ventricular aorta. The horns of the septum aorticum reach on the left side the commissure between cushions 3 and 4.

9 Pernkopf and Wirtinger failed to mention that a reduction in the musculature of the bulbus already occurs in amphibians and lung fish. Hence the second phase is really a further elimination of the bulbus rather than a separate process. However, we retain their terminology for convenience.

at ostium bulbotruncare and on the right side the anterior part of cushion 1. Thus in the reptile the truncus is divided into three vessels, two systemic and one relatively small pulmonic, while in the mammal it is divided into only two vessels, a systemic and a relatively larger (as compared with that of the reptile) pulmonic vessel.

The bulbus of the reptile and that of the mammal likewise show points of difference (fig 5). In both there are four cushions, 1, 2, 3 and 4, at the distal ostium of the bulbus and two cushions, A and B, at the proximal ostium. In both in an early stage of development there is also cushion C, situated to the right between A and B. However, this cushion disappears before the septums form in the bulbus. The difference between the two species lies in the ridge connections between the proximal and

septum is developed from ridges 1A and 3B. Thus are developed a right and a left aorta and a pulmonary artery in the reptile, and a single aorta and a pulmonary artery in the mammal.

#### POSSIBLE HYDRODYNAMIC EXPLANATION FOR THE ONTOGENETIC AND PHYLOGENETIC DEVELOPMENT OF THE HEART

We have thus traced the phylogenetic and ontogenetic development of the vertebrate heart. The understanding of the possible forces involved in this development we owe for the most part to Spitzer.<sup>6</sup> Although Robertson<sup>5</sup> some years previously had pointed out the correlation between the changes in the bulbus and the development of the lungs, it was Spitzer who first suggested the possible hydrodynamic principles involved in the changes in the circulation in the

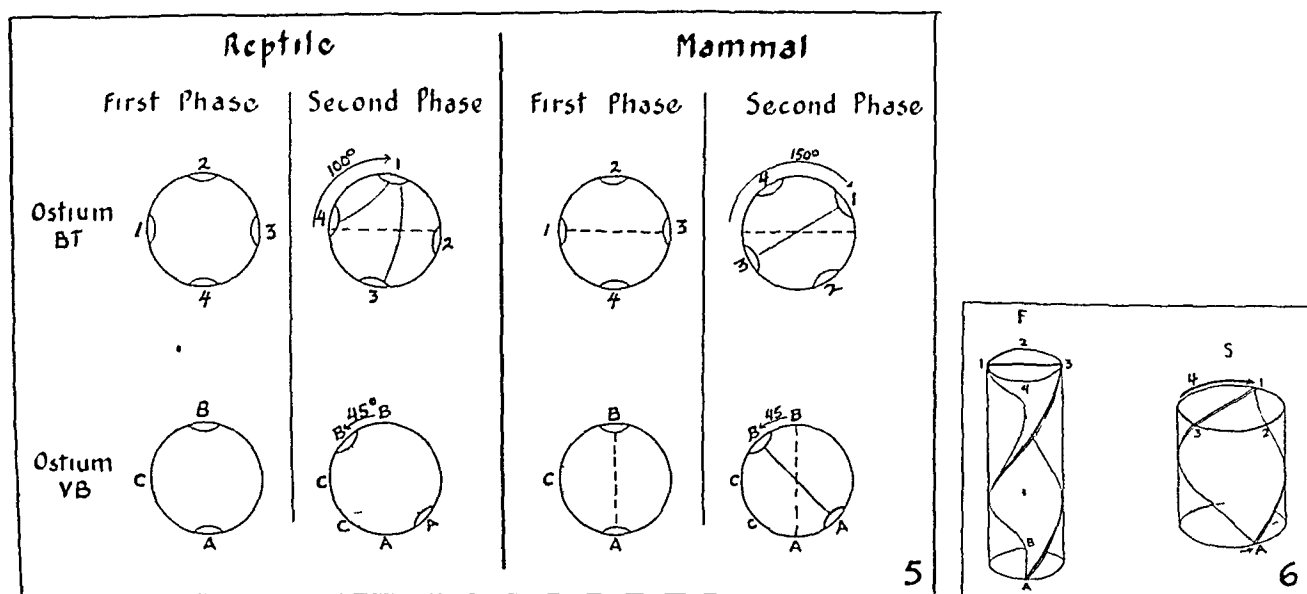


Fig 5—Comparison between the bulbus of the reptile and that of the mammal. Note that the number and the location of the bulbar cushions are the same. The difference lies in the number of ridges extending through the bulbus, connecting the distal and proximal cushions. There are three ridges in the reptile—ridges 1A, 4B and 3C. In the mammal there are only two ridges—1A and 3B. Therefore two septums are formed in the reptilian bulbus, but only one septum is formed in the mammalian bulbus.

Fig 6—Possible mechanical explanation for the absorption of the bulbus. F, first phase; S, second phase. 1, 2, 3, 4, distal bulbar cushions; A, B, proximal bulbar cushions. 1A and 3B, bulbar ridges.

distal bulbar cushions and the extent of their twist. In both, ridge 1A (homologous to the spiral valve of the Amphibia) is developed with a twist of 270 degrees. However, there are two further ridges in the reptile, 4B and a ridge going from cushion 3 to a point where cushion C originally was (we may call it 3C). In the mammal only a single opposite ridge 3B is developed. Neither of the ridges 4B and 3C has the twist of ridge 3B in the mammal. In the reptile are thus developed two septums in the bulbus—septum aortopulmonale from 1A and 3C and septum aorticum from ridges 1A and 4B, each continuous with the similar septums in the truncus. In the mammal, however, a single

phylogenetic series correlated with the development of the lungs. Utilizing the morphologic analysis made by Pernkopf and Wirtinger we have slightly extended Spitzer's conception. We present it in the following hypothesis, which is offered for the purpose of serving as an aid in the study of congenital anomalies.

The function of any circulation is the transportation and supply of materials for the metabolism of the component cells of the body and the removal of the waste products of metabolism. The differentiation of red blood cells during embryologic development and the simultaneous development of sinusoids and eventually of vascular tubing first to house and eventually to

transport the red blood cells thus may be correlated with the growth in number of the remainder of cells in the embryo and their further specialization necessitating and calling forth (by biologic forces as yet unknown) a circulation. Also, with greater specialization of body cells, the circulatory mechanism likewise becomes more specialized (by biologic forces as yet unknown), so that portions of the tubular structure take over the power of propulsion and hence the development in phylogeny and ontogeny of a straight tubular heart. When the circulation has reached a tubular stage, certain hydrodynamic principles may be assumed to act in its further development. The rapid increase in the number of systemic cells and the further specialization call forth a greater number of red blood cells and capillaries from the mesenchyme to house them. This increases the blood volume which in turn produces increased longitudinal growth of the heart tube and a telescoping in its relatively smaller containing cavity. The factor of the blood volume is possibly augmented by the factors of the pulse wave and the collision between the blood and the wall of the tube (Spitzer<sup>6</sup>). This telescoping produces the constructions at the sinoauricular, auriculoventricular and ventriculobulbar orifices (Spitzer). With further growth the tube begins to become kinked on itself. This is the stage reached by the elasmobranch and is rapidly telescopically recapitulated by higher vertebrates.

The bending and the torsion with the formation of the auriculoventriculobulbar loop and the bulbar bayonet are seen in the amniotic embryo and constitute the definitive form of the Dipnoi and the Amphibia. This formation is correlated with the development of the pulmonary circulation. The lungs represent a greater oxygen exchange mechanism than do the gills because of their relatively greater respiratory surface. This in turn is correlated with an increased capillary bed. The increase in number of "respiratory" capillaries is correlated with a marked increase in blood volume, and both are correlated in the phylogenetic development with the greater amount of oxygen in air as compared with sea and fresh water. The increase in blood volume produces a further increase in the size of the heart tube in comparison with its containing cavity, and thus it goes through the movements of the first phase with the accompanying torsions in multiple planes and the formation of a bayonet-like kinked bulbus. With the increase in blood flowing through the truncus into the pulmonary artery there are relative widening and shortening of the truncus. The centripetal force affects also the dividing spur between the fourth and sixth arches so that it is pulled

toward the heart tube. At the same time the spur is stimulated to growth because of the irritation produced by the bilateral pressure of the increased volume. Thus we have beginning septation in the truncus. A similar process occurs at the venous end stimulating the growth of the spur between the two venous ostia, leading to septation in the auricular regions. The same factors produce lateral distention and relative shortening of the dilated portions of the heart tube. The contracted portions between these dilated regions are thus pulled in the opposite direction with production of longitudinal folds. These are the distal and proximal bulbus swellings, the endocardial cushions and the sinoauricular folds. Thus terrestrial life is accompanied by the development of lungs, which is correlated with the development of the multiaxial auriculoventriculobulbar loop, the formation of the bulbar bayonet and the beginning of septation throughout the heart tube. These are the movements of the first phase resulting in partial septation of the circulations, with a twist of 180 degrees being imparted to the circulating blood. The development stops here in the Dipnoi and the Amphibia but goes on in the embryos of higher animals.

With still further development of the pulmonary circulation in amniotes, septation proceeds onward throughout the heart tube. The further increase in the blood going into the pulmonary artery produces a shortening of the bulbus and eventually in all amniotes its absorption, partial or complete, in the ventricles (fig 6). The centripetal force created by the increased pulmonary flow, acting on the truncus, acts on the curved bulbar ridges as well, eliminating most of their twist and handing this to the truncus. The result is torsion at the ostium bulbotruncare. The same increased force acting from the arterial side acts on the venous side. With increased flow through the pulmonary veins, the hydrodynamic force acting in the opposite direction to that coming from the arterial side produces a back torsion and ventral deviation of the bulbus (fig 3). The pulmonary flow in the reptile is less than that in the mammal and this accounts for the morphologic differences in the bulbus and the truncus and in the absorption of the bulbus in the two species. The pulmonary portion of the truncus in the reptile is relatively smaller than that in the mammal. This is due to the more posterior attachment of the horns of the septum aortico-pulmonale in the reptile as compared with the mammal, related to the smaller pulmonary flow. This permits the growth of the septum aorticum from the left caroticoaortic spur and brings about the adaptation of two aortas in present day reptiles.

In mammals the increased pulmonary flow, witnessed by the more anterior attachment of the septum aortico-pulmonale to cushions 1 and 3, inhibits the formation of the septum aorticum. The bulbus of the mammal likewise shows the greater importance of the pulmonary circulation by the growth of two opposite ridges instead of three and the production of a single septum aortico-pulmonale bulbi. The absorption of the bulbus of the mammal is more complete than that of the reptile, with the bulbar septum coming in line with the ventricular septum, completing the process of septation. The mesocardial portion of the bulbus, now being completely separated from the pulmonary portion, disappears or is telescoped into the left ventricle with complete absorption of the bulbus. The two circulations are now completely separated, and the efficiency of the pump has reached its highest point in the evolutionary development.

As mentioned earlier, in addition to the increase in efficiency of the pump, there was a second adaptation for the utilization of the high oxygen medium in the vertebrate series. This was the increase in the oxygen capacity of the blood. This was also a gradual evolutionary development, as seen in the accompanying table (Baldwin<sup>10</sup>).

	Cc of Oxygen per 100 Cc of Blood
Mammals	25.0
Birds	18.5
Reptiles	9.0
Amphibians	12.0
Fish	9.0

The increased amount of oxygen in the air, just as it must have served as the stimulus for changes in the lungs and in the pumping mechanism, must have served to increase the capacity of the blood for carrying oxygen. Hemoglobin is regularly present throughout the vertebrates and is the most efficient of oxygen-carrying pigments. Throughout the vertebrate series there is an increased morphologic differentiation of erythrocytes, coupled with an increased concentration of hemoglobin within them, accompanied by an increased number of them, all of which leads to greater oxygen carrying capacity (Redfield<sup>2</sup>). Because of the gradual increase of carbon dioxide produced by the cells in the vertebrate series, there ensued an increase of carbon dioxide-combining capacity and buffering capacity of the blood, which is seen in the evolutionary series accompanying the increase in hemoglobin. This property of carrying carbon dioxide, however, is not solely dependent on

hemoglobin, it is correlated with serum albumin, globulin and certain inorganic substances, which are dependent on processes in the kidneys and other organs (Redfield<sup>2</sup>).

An accompaniment of the increase in oxygen capacity of the blood was an adaptation for more rapid and complete exchange of oxygen between blood and tissue—increased oxygen tension. This tension is low in fish and reptiles but high in birds and mammals (Redfield<sup>2</sup>).

Thus, in summary, the phylogenetic and ontogenetic development of the heart is part of the whole development of the circulation in the phylogenetic tree. This is mostly related to the change in habitat of the vertebrates, from fresh water to land. The interaction between the respiratory and circulatory system of these animals and the high oxygen content of the environment led to the progressive evolutionary development of the mammalian circulation.

#### A THEORY OF TRANSPOSITION

Transposition of the arterial trunks has been defined by Abbott<sup>11</sup> as that condition in which "the great trunks have undergone an alteration in their relative position to each other or to the ventricles from which they emerge, whereby the aorta comes to lie in the path of the unaerated blood from the right ventricle." Thus within this category of anomalies lie those conditions in which the aorta emerges from both ventricles or from the right ventricle alone, and the pulmonary artery springs from the right or the left ventricle. Also included are those conditions of mutually abnormally situated arterial vessels arising from a common or a slightly subdivided ventricle, likewise, truncus communis, truncus solitarius aorticus and truncus solitarius pulmonalis, in which the aortic vessel or component emerges from the right ventricle, or the aorta or its remnant is abnormally situated with respect to the pulmonary artery or its remnant.

Transposition of the arterial trunks may be classified as follows:

Type I (Spitzer) Riding aorta (Rokitansky)

- (a) with an aneurysm of the membranous septum
- (b) with a defect of the ventricular septum

Type II (Spitzer) Partial transposition (Rokitansky)—The aorta and the pulmonary artery arise from the right ventricle.

Type III (Spitzer) Complete transposition (Rokitansky)—The aorta comes from the right ventricle and the pulmonary artery from the left ventricle.

Miscellaneous group Truncus communis, truncus solitarius aorticus with transposition, truncus solitarius pulmonalis with transposition, Type IV (Spitzer) and transposition with atresia of the tricuspid valve.

<sup>10</sup> Baldwin, E. An Introduction to Comparative Biochemistry, New York, The Macmillan Company, 1937.

<sup>11</sup> Abbott, M. E., in Osler, W. Modern Medicine, Philadelphia, Lea & Febiger, 1927.

All types of transposition of the arterial trunks may be considered to be due to a "phylogenetic" abnormality complicated by further "ontogenetic" abnormalities as postulated by Spitzer.<sup>6</sup> The phylogenetic abnormality is in our opinion an abnormality in the formation of ridge 3B. As pointed out, the normal mammalian bulbus presents two ridges, 1A and 3B, instead of the reptilian three ridges, 1A, 4B and 3C (fig 5). Ridge 1A has already been developed in the lung fish (fig 2). Ridge 3B, however, is a new formation not present below the mammal. When in the embryo of our reptilian ancestor the first responses to a greater increase in oxygen intake began, thus starting the mammalian series, there apparently was elaborated a fusion of ridges 3C and 4B by the already described hydrodynamic principles. It was possible, with the aforementioned forces, for the bulbus to be completely absorbed, with production of the mammalian heart. The processes involved in this absorption of the

most of the twist of the bulbar ridges and produced disappearance of the median bulbar segment, marked shortening of the bulbus, absorption of its ventral portion as the conus of the right ventricle and disappearance of the dorsal portion.

In the embryo whose parent form will present the anomaly transposition, although the stimulus is apparently there (as seen from the normal formation of lungs), it does not produce this new ridge 3B but produces one or the other or neither of its components, 4B and 3C. In other words it is doing what most of the reptilian embryos did during the time our pioneering reptilian ancestor made a progressive change. Yet the hydrodynamic forces for the absorption of the bulbus are there. The absorption of such a bulbus with a well formed ridge 1A but an abnormal ridge 4B and 3C, or a poorly developed ridge 3B, or with only ridge 1A, proceeds abnormally (fig 7). In the first place, the

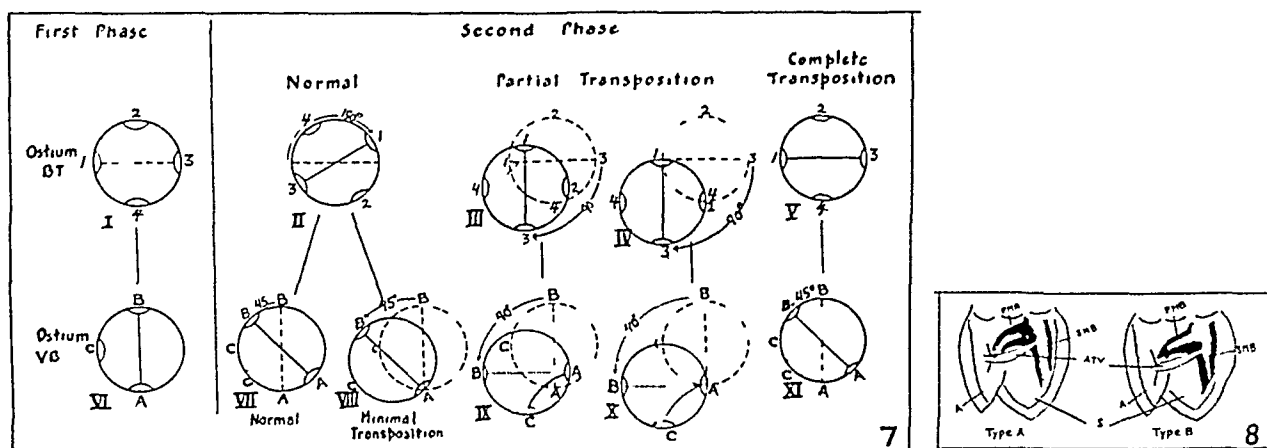


Fig 7—Normal and abnormal absorption of the bulbus. In transposition there is an abnormality in the formation of ridge 3B, whereby it is poorly formed or replaced by ridge 4B or 3C. This results in decreased torsion at the distal ostium. In addition, this torsion occurs either around cushion 1 as a center or close to cushion 1. At the proximal ostium back torsion occurs around cushion A as a center or close to cushion A. This back torsion may be 45 degrees, or it may be 90 degrees as is normally observed in an earlier stage, 1, 2, 3, 4, distal bulbar cushions A, B, C, proximal bulbar cushions BT, ostium bulbotruncare VB, ostium ventriculobulbare I, VI, distal and proximal bulbar ostia, respectively, at the end of the first phase (normal and in transposition). II, VII, distal and proximal bulbar ostia during the second phase in normal absorption of the bulbus. III, IX, bulbar ostia in partial transposition during the absorption of the bulbus. IV, X, bulbar ostia in partial transposition during the absorption of the bulbus when torsions occur about cushions 1 and A as centers. V, XI, bulbar ostia in complete transposition in which no torsion occurs at the distal ostium while mild back torsion occurs at the proximal ostium.

Fig 8—Topography of the muscle bundles of the right ventricle. In Type A two distinct muscle bundles are noted. In Type B an arch of musculature is present at the base of the pulmonic valve. SMB, septal muscle bundle; PMB, parietal muscle bundle; ATV, anterior leaflet of tricuspid valve; A, anterior wall of the right ventricle; S, septal wall of the right ventricle.

bulbus were a torsion of 150 degrees (counterclockwise—looking truncusward from the bulbus) at the ostium bulbotruncare and a back torsion of 45 degrees (clockwise—looking bulbusward from the ventricle) at the ostium ventriculobulbare (fig 3). The latter was combined with a deviation directed ventrally and to the left of the bulbus. These processes eliminated

torsion at the distal and proximal ostia cannot occur around the center of the tube as an axis, the center of rotation will be about a point between the center of the tube and cushions 1 and A, or on the periphery at cushion 1 and cushion A. In addition there will be less torsion at the distal ostium. For since we believe the torsion to be produced by the action of the centripetal

force on the spiral ridges, the presence of only one ridge with or without an opposite ridge with a lesser twist will lessen the torsion. The torsion of the proximal ostium may remain the same but will be eccentric with its center closer to A or at A. Thus the conuses of the aorta and the pulmonary artery will take up abnormal positions after the absorption of the bulbus. Hence the ontogenetic complication of the phylogenetically abnormally formed bulbar ridges is the abnormal absorption of the bulbus.

#### TYPES OF TRANSPOSITION

We may now briefly discuss the various types of transposition and their underlying abnormalities.

associated with a mild degree of transposition. This was based on a study of the muscle bundles of the right ventricle in addition to the abnormal position of the aorta. In our opinion the underlying variant here is a poorly formed ridge 3 B resulting in a slight abnormality in the absorption of the bulbus. That is, normal torsion occurs at the distal ostium but slightly diminished back torsion occurs at the proximal ostium (II and VIII in fig 7). This results in poor fusion of the proximal cushion of the bulbus, the endocardial cushions and the ventricular septum, leading to an aneurysm in the resultant pars membranacea.

*Type 1B—Riding Aorta with a Defect of the Ventricular Septum* (fig 9b). Here the aorta as in the previous type, straddles the muscular

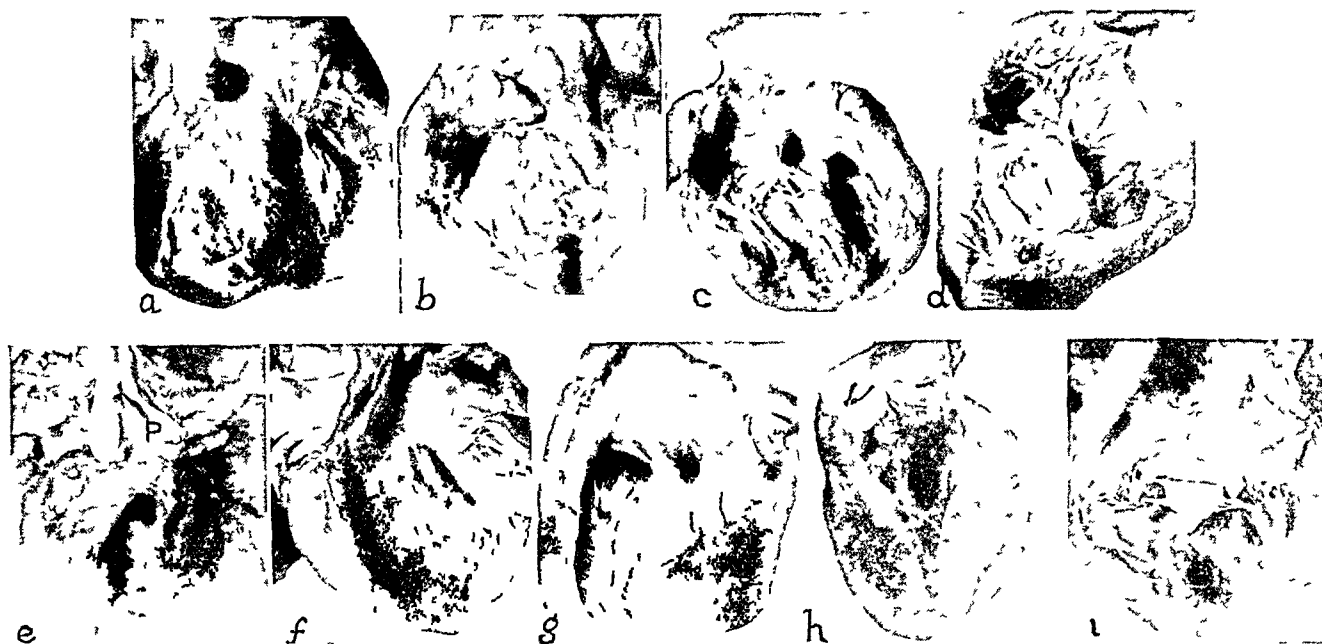


Fig 9—(a) Type 1A. This is a riding aorta with an aneurysm of the membranous septum, left ventricular view. (b) Type 1B. This is a riding aorta with a defect of the ventricular septum, right ventricular view. (c) Partial transposition with the tetralogy of Fallot, right ventricular view. (d) Partial transposition with the tetralogy of Eisenmenger, right ventricular view at the base of aorta. (e) Partial transposition with the tetralogy of Eisenmenger, right ventricular view at the base of the pulmonary artery (P, the pulmonary artery). (f) Complete transposition. (g) Truncus arteriosus communis persistens, right ventricular view. (h) Transposition with atresia of the tricuspid valve as it appears to one looking into the heart anteriorly. (i) Transposition with atresia of the tricuspid valve as it appears to one looking into the heart posteriorly.

*Type 1A—Riding Aorta with Aneurysm of the Membranous Septum* (fig 9a).—In this type the aorta straddles the muscular ventricular septum over an aneurysm in the obliquely situated pars membranacea. The aorta is thus shifted slightly anteriorly, but the coronary arteries and ostia show no evidence of transposition. More than 70 cases have been reported in the literature (Lev and Saphir<sup>12</sup>), in about 15 per cent of which the abnormal position of the aorta was noted. In our previous report on this anomaly we showed that congenital aneurysm of the membranous septum is often (if not always)

ventricular septum, but instead of an aneurysm of the pars membranacea there is a defect of the ventricular septum. The aortic semilunar cusps are displaced in a slight counterclockwise direction (looking from the aorta toward the ventricle). The coronary arteries show an abnormal course typical of mild transposition (see Spitzer<sup>6</sup>). Stenosis of the pulmonary ostium with a bicuspid pulmonic valve may be present.

In our opinion the underlying abnormality here is a poorly formed ridge 3 B, this ridge being less in extent than in congenital aneurysm of the membranous septum. This results in abnormal absorption of the bulbus as follows: less than the normal 150 degrees of torsion in

<sup>12</sup> Lev, M., and Saphir, O. Arch Path 25:819, 1938.

the distal ostium and eccentric distal bulbar torsion and proximal bulbar back-torsion, as indicated in the previous type (*III* and *IX* in fig 7) Ridge 3 B does not meet the ventricular septum, thus preventing the completion of the latter, with the result that there is a defect in the anterior portion of the ventricular septum

*Type II—Partial Transposition* (fig 9 c, d and e)—In this type the aorta emerges from the right ventricle with the pulmonary artery. No vessel emerges from the left ventricle which presents the defect in the ventricular septum as its only outlet. The aortic semilunar cusps are still more rotated in a counterclockwise direction. The coronary arteries show a greater degree of transposition than in the previous type. The pulmonary orifice and trunk (tetralogy of Fallot) (fig 9 c) or the aortic orifice and trunk (tetralogy of Eisenmenger) (fig 9 d and e) (Saphir and Lev<sup>13</sup>) may be hypoplastic. As in the previous type there is always a defect of the ventricular septum.

In our interpretation the abnormality here is absence of ridge 3 B and its replacement by ridge 4 B (in the tetralogy of Eisenmenger) or 3 C (in the tetralogy of Fallot). The absorption of such a bulbus proceeds abnormally as follows: less torsion at the distal bulbar ostium and normal or 90 degree torsion at the proximal bulbar ostium. These torsions may occur with cushions 1 and A as centers (*IV* and *X* in fig 7) or with a point between the center of the bulbar tube and cushions 1 and A as centers (*III* and *IX* in fig 7). The abnormal ridge produces in addition a narrowing of either the pulmonary or the aortic tract.

*Type III—Complete Transposition* (fig 9 f)—In this anomaly the aorta emerges from the right ventricle and the pulmonary artery from the left. In most cases the ventricular septum is closed. In a lesser number there is a defect in the anterior portion of the ventricular septum. The arterial trunks ascend from the heart almost parallel to each other. The ostia of the coronary arteries are usually more transposed, being situated in the posterior sinuses of Valsalva. The coronary arteries are still more abnormal. The thickness of the right ventricle exceeds that of the left. The remainder of the heart is usually normal.

In our interpretation the abnormality here is complete absence of ridge 3 B with no replacement. Ridge 1 A has been destroyed by the abnormal currents created in such a bulbus. The bulbus has then been absorbed without torsions

(*V* and *XI* in fig 7). At the end stages of absorption of such a bulbus currents are established which lead to the production of a proximal bulbar septum, thus completing the ventricular series.

*Type IV—Miscellaneous Group*—In this group are included Spitzer's group IV, truncus communis (fig 9 g), truncus solitarius aorticus, truncus solitarius pulmonalis with transposition and transposition with atresia of the tricuspid valve (fig 9 h and i). Spitzer's group IV consists of those cases in which a hypoplastic aorta emerges from a small humplike pouch, separated from a large ventricular space into which open both auriculoventricular ostia, or a common auriculoventricular ostium, and from which emerges the pulmonary artery.

In truncus communis there is not only an abnormality in the absorption of the bulbus as indicated in types I and II but an abnormality in the septum aorticopulmonale trunci (Lev and Saphir<sup>14</sup>). Truncus solitarius aorticus and truncus solitarius pulmonalis are exaggerated forms of type II transposition (Eisenmenger or Fallot types). In transposition with atresia of the tricuspid valve one is dealing with an absence of ridge 3 B, but ridge 1 A is not obliterated. The absorption of a bulbus with such a ridge proceeds as follows. At the distal bulbar ostium less torsion occurs, while at the proximal ostium there is 45 to 90 degrees of back torsion. Because of the absence of ridge 3 B, torsion occurs about ridge 1 A as a center (*IV* and *X*). In addition there is an abnormality in the auriculoventricular ostia. Normally, besides the bulbar movements occurring in the second phase, there is a shift of the auricular canal to the right (fig 4). Thus, whereas both auriculoventricular ostia are originally to the left side of the bulbus at the end of the first phase, the tricuspid orifice passes to the right of the mesocardial portion of the bulbus after the normal movements of the second phase. With this goes an expansion of the tricuspid orifice and of the dorsal wall of the meta-ampule (distal portion of the primitive ventricle). If, therefore, there is an abnormality in the absorption of the bulbus, especially in the type in which rotation occurs about ridge 1 A as a center, then in some cases the tricuspid orifice and the right auricle do not reach their normal definitive position to the right of the bulbus. This results in atresia of the tricuspid valve.

The abnormality in Spitzer's type IV is similar to that just described without the changes in the auricular canal.

<sup>13</sup> Saphir, O., and Lev, M. Am Heart J 21 31, 1941

<sup>14</sup> Lev, M., and Saphir, O. J Pediat 20 74 1942



## CRITERIA FOR THE PATHOLOGIC DIAGNOSIS OF TRANSPOSITION

The criteria for the presence of transposition are (1) an abnormal position of the arterial trunks, (2) an abnormal position of the ostiums of the coronary arteries (counterclockwise displacements if one is looking from the aorta into the ventricle), (3) an abnormal topography of the muscle bundles of the right ventricle and (4) an abnormal distribution of the coronary arteries.

The distribution of the coronary arteries has been thoroughly studied by Spitzer.<sup>6</sup> The ramus descendens anterior is taken over by the artery arising from the right sinus of Valsalva, or two branches, one from the right and one from the left, may be present. The posterior descending branch is usually taken over by the artery arising from the left sinus of Valsalva. In some cases the two sinuses may have completely changed branches.

The topography of the muscle bundles of the right ventricle in the normal heart and that in the heart with transposition have been studied by many, and their work is reviewed elsewhere.<sup>1</sup> In our studies we noted two types of topography prevalent in normal hearts (fig 8). In most cases two distinct muscle bundles are noted. From the septal cusp of the pulmonic valve a muscle bundle obliquely descends over the septum toward the apex. This we have called the septal muscle bundle or band. Near the apex it gives off the moderator band. On the anterior wall of the right ventricle the parietal muscle bundle or band ascends toward the base of the heart in close proximity to the anterior leaflet of the tricuspid valve. The superior portion of the muscle terminates at the septal pulmonic cusp. Its main intermediary portion fuses with the musculature of the septum beneath the septal band. A distinct raphe is noted where

the parietal band dips behind the septal band. The inferior portion terminates at the base of the anterior leaflet of the tricuspid valve. In a smaller number of cases there is a mass of musculature forming an arch over the base of the ventricle at the base of the septal cusp of the pulmonic valve, with fibers radiating over the anterior wall of the right ventricle, adjacent to the tricuspid valve and down over the right wall of the septum. This formation apparently represents a fusion of the septal and parietal bands. From the evidence of Keith,<sup>4</sup> and Pernkopf and Wirtinger,<sup>7</sup> the formation of the parietal band is related to the formation of the proximal bulbar septum and correlated with the growth of ridge 3 B in mammals. It is thus derived from cushion B, the bulboauricular spur and the counter ridge B<sub>0</sub> of the ventricular septum. The septal band, from the evidence of Spitzer,<sup>6</sup> Fuchs,<sup>15</sup> Benninghoff,<sup>15</sup> Tandler,<sup>16</sup> and Keith,<sup>4</sup> is derived from cushion A (or is an extension of bulbar ridge 1 A).

In transposition of the arterial trunks the septal band is usually hypertrophied and may even form a pseudoseptum (Spitzer). The parietal muscle bundle is usually diminished in size or may be completely absent.

## CONCLUSION

Transposition of the arterial trunks may be considered to be produced by an abnormality in the absorption of the bulbus, in the second phase of the development of the heart. This we believe is due to an abnormality in the phylogenetically recently developed bulbar ridge 3 B.

15 Cited by Pernkopf and Wirtinger.<sup>7</sup>

16 Tandler, J., in Keibel, F., and Mall, F. P. *Manual of Human Embryology*, Philadelphia, J. B. Lippincott Company, 1912, vol. 2.

## PARAPHYSIAL CYST OF THE THIRD VENTRICLE

LIO D. MOSS, M.D., OLEAN, N. Y.

The paraphysis is a phylogenetically ancient organ which in former years interested mainly zoologists and embryologists. It was first described by Selenka,<sup>1</sup> in 1890, who found it in embryos of various vertebrates. The gland is best developed in Amphibia. In adults of this class it occurs as a well developed organ. Its location is the anterior portion of the roof of the third ventricle. The paraphysis of *Necturus*, a member of the class Amphibia, was studied in great detail by Warren,<sup>2</sup> who described it as a lobulated organ composed of anastomosing tubules. The function of this organ is apparently not known. However, it may be mentioned that some authors<sup>3</sup> have accepted a certain homology between the paraphysis of vertebrates and the acoustic organ of ascidians better known under the popular name "sea squirts". The ascidians (tunicates) represent an interesting group of nonvertebrates which during the larval state of their existence display certain features peculiar to vertebrates, such as a chorda dorsalis and a tubular nervous system. In vertebrates above Amphibia the paraphysis becomes gradually less conspicuous. Rudiments of this organ in the human embryo were first described by Francotte,<sup>4</sup> in 1894. As early as 1909 it was suggested by Sjovalld<sup>5</sup> that remnants of the paraphysis may give rise to cysts. In recent years such cysts of presumably paraphysial origin have been described in increasing number, and up to date almost 70 cases have been reported in the literature. With but few exceptions,<sup>6</sup> most of the cases reported in this country appeared in publications pertaining to neurology,<sup>7</sup> although the general pathologist is

apt to encounter such cysts in routine autopsy material. An additional case is reported here.

## REPORT OF A CASE

The patient was a woman 29 years old. She was seen late one evening by Dr. H. G. Storer, Olean, N. Y., at her home. She complained of excruciating headaches associated with spells of vomiting. These headaches had begun late the same afternoon. Her pain was severe enough to necessitate two subcutaneous injections of a solution of 1 morphine salt during the night. The physician made a tentative diagnosis of intracranial neoplasm. The patient died the following morning. Information obtained from relatives disclosed that she had suffered from attacks of headaches for a long time and that they had increased in frequency and severity following extraction of a tooth two and a half months prior to her death.

*Postmortem Examination* (three hours after death).—The organs of the thoracic and abdominal cavities did not disclose any pertinent abnormalities. The brain revealed an approximately normal amount of subarachnoidal fluid. The pia-arachnoid was not thickened. The gyri appeared to be slightly flattened. Coronal sections (fig. 1) of the brain showed marked dilatation of both lateral ventricles. The degree of dilatation was about equal on both sides. In the vicinity of the interventricular foramina of Monro, the roof of the third ventricle presented a cystic mass which extended downward into the cavity of the third ventricle. This cyst measured 1.5 cm. in diameter and contained grayish white viscid fluid. The septum pellucidum above it was extremely thin. The cyst leaned against the foramina of Monro, obviously obstructing the latter. The anterior portion of the third ventricle was enlarged by the cyst, which was suspended from its roof. The aqueductus cerebri and the fourth ventricle were not dilated.

*Microscopic Examination of the Cyst*.—In addition to the main cyst there were several smaller cystic areas as seen in a multiloculated cyst. The linings of all these cystic areas were about the same (figs. 2 and 3). They consisted of one or two layers of cuboidal cells, some of which were quite flat. A number of the lining cells were distinctly ciliated. The nuclei were fairly large in comparison with the size of the cells and were usually at the base of the cells, away from the ciliated surface. The cytoplasm frequently showed various degrees of vacuolation. Where the vacuolation was extreme, cilia could not be discerned. Indeed, some of these cells had no sharp cell boundaries toward the free surface and appeared to merge with the contents of the cyst. The latter stained moderately acidophilic. Several small concretions which stained bright red with eosin were present in the cyst contents. They were surrounded by polymorphonuclear leukocytes and a few desquamated epithelial cells. Underneath the lining epithelial cells

From the Department of Pathology, Olean General Hospital.

- 1 Selenka, E. *Biol. Centralbl.* **10**: 323, 1890-1891.
- 2 Warren, J. *J. Comp. Neurol.* **28**: 75, 1917.
- 3 Krabbe, H. K. *Brain* **59**: 483, 1936. Selenka<sup>1</sup>.
- 4 Francotte, P. *Bull. Acad. roy. d. sc. de Belgique* **27**: 84, 1894.
- 5 Sjovalld, E. *Beitr. z. path. Anat. u. z. allg. Path.* **47**: 248, 1909.
- 6 (a) Rehbock, D. J. *Arch. Path.* **21**: 524, 1936. (b) Weinberger, L. M., and Boshes, B. *Surgery* **13**: 368, 1943. (c) Hassin, G. B., and Anderson, J. B. *U. S. Vet. M. Bull.* **6**: 56, 1930.
- 7 (a) Dandy, W. E. *Benign Tumors of the Third Ventricle of the Brain*, Springfield, Ill., Charles C. Thomas, Publisher, 1933. (b) Zimmerman, H. M., and German, W. J. *Arch. Neurol. & Psychiat.* **30**: 309, 1933. (c) Rinder, C. O., and Cannon, P. R. *ibid.* **30**: 880, 1933. (d) Stookey, B. *Bull. Neurol. Inst. New York* **3**: 446, 1934. (e) Davidoff, L. M., and Dyke, C.

- ibid.* **4**: 221, 1935. (f) McLean, A. J. *Arch. Neurol. & Psychiat.* **36**: 485, 1936. (g) Gardner, W. J., and Turner, O. *ibid.* **38**: 1055, 1937. (h) Shaver, M. R. *ibid.* **43**: 510, 1940. (i) Zeitlin, H., and Lichtenstein, B. *J. Nerv. & Ment. Dis.* **91**: 704, 1940. (j) Larson, C. P. *ibid.* **91**: 557, 1940.

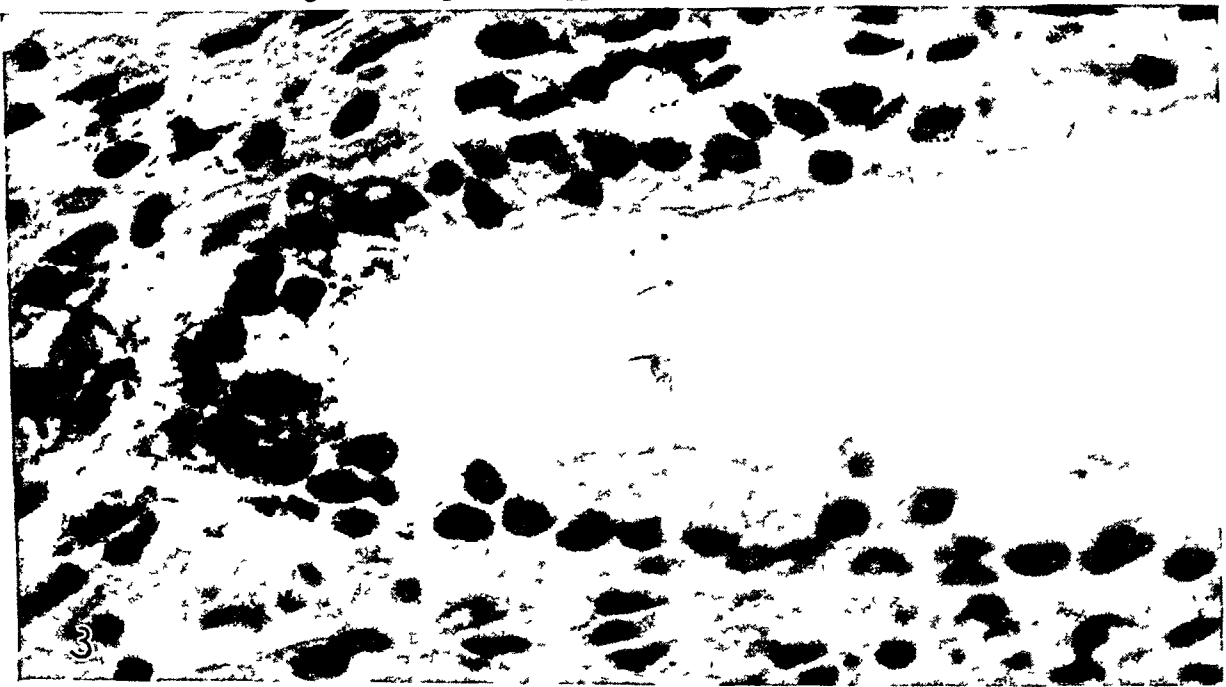


Fig 1—Coronal section of the brain showing a cystic mass filling the dilated anterior portion of the third ventricle. Note the marked dilatation of the lateral ventricles.

Fig 2—Cyst wall under low power magnification.

Fig 3—High power field (dry) showing the lining of one of the additional smaller cysts with partly ciliated epithelium and large macrophages in the adjacent stroma.

there was a stroma which consisted in part of collagenous tissue, varying in thickness, and in part of densely packed, fairly large cells containing clumps of light grayish brown pigment. These cells did not take a sudan stain or a stain for iron. They apparently were macrophages. In one area there was a small tubule lined by cuboidal epithelium. Such tubules, as well as the previously mentioned additional small cystic areas, were thought by Zeitlin and Lichtenstein<sup>71</sup> to be proof of origin in the paraphysal anlage. Most of the external surface of the cyst had lost its epithelial lining. In some areas, however, one could still discern a single layer of low cuboidal epithelium such as that seen in the ependyma. In other sections the choroid plexus was observed to be attached to the outer surface of the cyst wall. The adjacent tissue of the brain (columnae fornicis) showed a few minute areas of fresh hemorrhage.

#### COMMENT

The clinical importance of such a cyst lies in its strategic position near the foramina of Monro. Acute impaction in the foramina has been mentioned as a cause of sudden death and was probably the cause of death in the case now described. At the same time the close relationship of such a cyst to the choroid plexus is noteworthy. Indeed, some cysts of apparently paraphysal origin have been reported as choroidal cysts. However the present case illustrates again, as emphasized by other authors, that the cyst while expanding downward into the third ventricle becomes only enfolded by the choroid plexus without actually being part of, or having derived from, the same. Furthermore, Zeitlin and Lichtenstein pointed out that similar cysts containing colloid material are not found in the lateral and fourth ventricles, a fact which can be upheld effectively against the possible origin

of such a cyst of the third ventricle from the choroid plexus.

Cysts of this nature—also referred to as neuroepithelial cysts or colloid cysts of the third ventricle—have been described mainly in persons from 20 to 50 years old. They have been three times as frequent in males as in females. About half of all the patients whose cases have been reported died within one year after the onset of symptoms unless they were successfully operated on. The most important symptom is severe paroxysmal headache, frequently altered by a change of the position of the head. It has been suggested that these intermittent headaches are due to temporary encroachment on the foramina of Monro by a more or less freely suspended cyst. Such headaches are frequently accompanied by nausea as well as by papilledema. Stookey<sup>74</sup> expressed the belief that the headaches are produced by compression of both veins of Galen at their origin with coincident interference with the venous outflow of the choroid plexus of the lateral and third ventricles. Stookey<sup>74</sup> mentioned further that among patients examined more thoroughly symptoms referable to the diencephalon were found fairly frequently, such as hypersomnia, diabetes insipidus, hyperthermia, adiposity, vasomotor disturbances and others. Ventriculography has been helpful in establishing a diagnosis, as reported by Dandy<sup>7a</sup> and Davidoff and Dyke<sup>7c</sup>. Once diagnosed, these cysts are amenable to surgical intervention. Successful removal of such cysts with cure of the patients has been reported by a number of authors. Weinberger and Boshes<sup>6b</sup> reviewed the literature in 1943.

# General Reviews

## ARTERIOSCLEROSIS

W C HUEPER, M D

NEW YORK

## THE ANOXEMIA THEORY

(Concluded from page 131)

*Excessive Intake of Cholesterol*—The role of alimentary hypercholesteremia in the production of atheromatosis and atherosclerosis is not entirely well established. While the gradual development of persistent and considerable hypercholesteremia in herbivorous animals, especially rabbits, having normally a low level of blood cholesterol, is regularly seen when these animals are put on an abnormal diet containing appreciable amounts of cholesterol, the hematic reactions observed in this respect in carnivorous and omnivorous animals placed on a similar diet are distinctly different. Burger and Winterseel, as well as Burger and Habs, observed in a human subject given 5 Gm of cholesterol in olive oil a transitory hypercholesteremia reaching its peak within two hours after the oral introduction of the cholesterol. Similar observations of a transient increase of the blood cholesterol in man after the ingestion of a food rich in fats and cholesterol have been reported (Muller, Barreda, Gardner, Garb, Snapper and Parisel). Corcoran and Rabinowitch stated, on the other hand, that Eskimos living on a meat diet and having normally a high metabolic rate have normal blood lipid values. Page cited Tolstoi as having found that white men on a similar diet over long periods exhibited little change in the level of the blood cholesterol. In proper evaluation of these observations it must be noted that the Eskimos consume relatively little fat (Thomas), and thus there is no valid reason why alimentary hypercholesteremia should develop.

Okey and Stewart, on the other hand, observed in women after administration of a food containing large amounts of cholesterol (egg yolk, butter) a transitory increase in blood cholesterol. Similar observations in man fed powdered egg yolk were recorded by Steiner and Domański. The cholesterologenic nature of this reaction was demonstrated by the observation that the feeding of soybean lecithin to man caused hypocholesteremia. Flock, Corwin and Bollman obtained sustained hyperlipemia of dietary origin in dogs

Steiner and Domanski also noted such an effect in dogs given a cholesterol-rich food, but the elevation of the blood cholesterol remained within moderate limits and atheromatous reactions were absent. Li, Hough, Monahan and Freeman demonstrated in dogs fed a protein-deficient, high fat-cholesterol diet an increase of the serum cholesterol of 172 to 225 per cent. This reaction was much less marked when fat and cholesterol were given with a diet containing adequate amounts of protein. Reports of persistent alimentary hypercholesteremia in dogs and rats fed a cholesterol-rich diet were published by Corwin and Sperry and Stoyanoff. Whether or not the mildly and persistently increased blood cholesterol not infrequently noted in obese children and adults is of dietary or of endocrine origin has as yet not been determined (Ricketts). The available evidence indicates that the prolonged and excessive intake of cholesterol can elicit persistent, though usually mild to moderate, hypercholesteremia in omnivorous and carnivorous animals.

The earliest manifestation of an interrelation between lipoidal intimal deposits and an alimentary imbalance of the plasma cholesterol is probably represented by the so-called lipid spots of the aorta appearing as early as two months after birth (Ernst, Gorog). These spots and streaks apparently are responses to the relative hypercholesteremia elicited by a milk diet in an organism with an incompletely developed cholesterol metabolism. This conclusion is derived from the fact that the serum cholesterol level of the newborn is low (about 60 mg per hundred cubic centimeters) (Gyorgy, Hueck, Muhlbock and Kaufmann, Sperry), which contrasts sharply with the hypercholesteremia prevailing in the maternal blood during pregnancy, when the placenta apparently serves as a filtration membrane and a storage organ for cholesterol (Versé). The blood cholesterol of the infant approximately doubles during the first four months of life (Kube and Sslowjew, Knauer) and then rises grad-

usually until it reaches the normal serum cholesterol level of 130 to 230 mg per hundred cubic centimeters. Thus, according to the figures given before, the human infant behaves in regard to the alimentary intake of cholesterol, which is not inconsiderable, much as does the herbivorous rabbit, in which the serum cholesterol level remains at a low level (50 to 100 mg per hundred cubic centimeters) throughout life. Studies of Sslowjew on suckling rabbits have shown the presence of similar lipoidal deposits in the aortic intima and media, while these were absent in young rabbits which had been on a vegetarian diet for a period of one to two months.

The lipid spots and streaks observed in the human aorta and the large elastic arteries increase in frequency and extent during the first two decades and tend to disappear thereafter (Lubarsch, Saltykow, Monckeberg, Schmidtmann, Scheel, Zinserling, Kube and Sslowjew). They are present in all children above the age of 5, according to Schmidtmann. Similar changes develop during the same period in the ventricular side of the large mitral leaflet (Marius). The spots and streaks appear as white or white-yellow elongated formations, resembling drops running down a candle, and are not elevated or only slightly elevated above the intimal surface. They first develop just above the aortic cusps, later around the orifices of arteries branching from the aortic arch, in the scar of the ductus arteriosus, on the posterior wall of the aorta, either around or at the lower edges of the orifices of the intercostal and lumbar arteries or between the orifices of the intercostal arteries at the posterior aspect of the aortic wall, at orifices of the large abdominal arteries and, finally, in the lower abdominal portion of the aorta, affecting also the lateral and anterior aspects (Zinserling, Ranke, Lange, Froboese). The lipoidal material appears first in the endothelial cells and extracellularly in the subendothelial layer, but usually also here within histiocytes, especially when little ground substance is present (Zinserling). Fibroblastic proliferation of a mild degree often accompanies these changes (Lubarsch, Schmidtmann). The lipid spots do not show any progressive degeneration and apparently are reversible (Thorel, Krisch). The cellular proliferation in the periphery of the lipoidal deposit may regress with the ultimate disappearance of the lipoidal material, sometimes leaving a minor focal hyaline thickening of the intima (Zinserling). During middle age both lipid spots and atherosclerotic lesions may co-exist and may even be superimposed on one

another (unless such combinations are to be considered different stages of the same process).

A lively controversy still exists concerning some aspects of the causation and the significance of these lesions, especially in regard to whether they are precursors of the atherosclerotic lesions developing in later decades. Beitzke asserted that the lipid spots are the result of defects in the intima caused by a transitory excessive dilatation of the aortic wall which elicits ruptures in the delicate and unyielding intima. Others (Askanaazy, Sanders, Zinserling, Simnitzky) related these lesions to toxic injuries sustained during infectious diseases. Still others (Saltykow, Ranke, Lubarsch, Benda, Anitschkow, Monckeberg, Kohlhaas, Oberndorfer, Busch, Schmidtmann, Zinserling) regard the lipid spots as early stages of atherosclerotic lesions, with which they share the same topographic distribution. Ranke claimed that gradual transitions between the lipid spots and typical atheromas can be demonstrated. Aschoff distinguished sharply between the atherosclerosis of infants and juvenile persons and the sclerotic atherosclerosis of adults. Others (Ribbert, Hueck, Lange, Cramer, Askanaazy, Froboese, Westenhofer, Jores, Beitzke) considered lipid spots as distinct from atherosclerosis for a number of reasons, such as the difference in the age of occurrence, the irreversibility of atherosclerotic lesions, the presence of productive and degenerative processes in atherosclerotic lesions, the greater distribution of the atherosclerotic changes in the arterial tree and the relative rarity of typical atherosclerosis in children (Benda, Seitz, Chiari, Filatoff and Rachmaninoff).

In the light of the evidence available on the genesis of lipoidal intimal deposits it appears that the differences between lipid spots and typical atheromas are not fundamental but mainly those of degree and duration. The causative agent and mechanism acting in the production of these manifestations during childhood act evidently with minor intensity and over a limited length of time, while those operative during adult life act through the course of decades and with greater strength.

The available evidence connecting an excessive alimentary intake of cholesterol-containing food-stuffs with the development of atherosclerosis in adults is mainly of circumstantial character (Beneke). Rosenthal, who compiled the data from twenty-eight papers dealing with the relation between diet and the incidence of atherosclerosis in various countries and under different climates, found that a diet high in fat content was almost invariably accompanied by a high

rate of atherosclerosis among its consumers, while diets with a low fat content, such as those consumed by Eskimos and Japanese, were usually associated with a low incidence of atherosclerosis. Investigations of Rubner (in Japanese) and Raab on this subject led to similar conclusions. Grotel, Bykhovskaya, Pavlova, Pokhodilova and Shoi found that the degree of atherosclerosis increased parallel with the quality of nutrition, being most pronounced in persons with excessive nutrition, particularly of cholesterol. Rosenthal stated that the intake of large amounts of neutral fat paves the way for the absorption of cholesterol through the intestinal wall. Cramer recorded an unusual incidence of intimal lipoidosis in tuberculous patients kept on a high fat diet. Additional support for the occurrence of alimentary atherosclerosis in man is supplied by the observations of Kuczynski among the inhabitants of the Kirghiz steppe. Kuczynski found that the nomads inhabiting the plain, who daily consume enormous amounts of mare's milk (20 liters) and meat (10 to 20 pounds [4.5 to 9 Kg]), showed in addition to obesity signs of precocious cholesterosis (extensive atherosclerosis, arcus senilis of the cornea), while the inhabitants of the towns, who consume a more moderate and mixed diet, exhibited a sclerosing hyperplastic type of vascular disease without regressive intimal changes.

Many attempts have been made to prove or to disprove the existence of relations between the cholesterol content of the blood and the development of atherosclerosis by comparing the blood cholesterol content with the condition of the arteries. The results obtained were contradictory, as might be expected from the fact that atherosclerosis is in general a process which takes years to develop and which may persist for many more years after the cessation of the action of the causal agent. The determination of the blood cholesterol content, on the other hand, merely renders information about the momentary condition of the blood, which may be entirely unrelated to the status of the blood prevailing when the vascular lesions were produced. Liebig thus found hypercholesteremia in 77 per cent of his patients with atherosclerosis and essential hypertension and concluded from this observation that cholesterol contributed to the development of the vascular lesions. Steiner and Domanski recorded for patients with coronary arteriosclerosis a serum cholesterol level which fluctuated between 308 and 499 mg per hundred cubic centimeters, average 355 mg, against values of 214 to 334 mg per hundred cubic centimeters, average 255 mg in a normal control group.

Davis, Stern and Lesnick reported similar observations in patients with angina pectoris and arteriosclerosis, many of whom showed an increase of all lipid fractions.

Numerous attempts have been made to reproduce atherosclerosis in carnivorous (dog, cat) and omnivorous animals (rat, mouse, chicken) by feeding a diet to which considerable amounts of cholesterol were added. Carnivorous animals normally have retention hypercholesteremia only during pregnancy and after castration (Lowenthal, Neumann and Heirmann, Roemer, Berberich, DaBella, Mancke). Omnivorous and carnivorous animals, like man, absorb and excrete rapidly ingested lipid material and therefore react with a rapid and transient rise of blood cholesterol (Yuasa). If lipid is fed over long periods, there is a decrease in the degree of visible lipemia as the organism becomes trained in handling lipids (Lowenthal). A combined protein-lipid diet increases the digestive transitory lipemia in these animals (Bang). While attempts to produce persistent hypercholesteremia in dogs by feeding cholesterol or lecithin in the diet, have been successful (Corwin, Grigaut and L'Huilier, Anitschkow), all efforts to elicit atheromatosis in these animals have so far failed (Wacker and Hueck, Hueck, Kawafuma, Adler, Anitschkow). Similar failures were obtained with cats (Cook, Yuasa, Hueck, Cilio, Anitschkow).

The experiments with rats fed a cholesterol-rich diet gave variable results. Numerous observers (Chalatow, Mosebach, Anitschkow and Chaladow, Cook and McCullagh, Chanutin and Ludewig) reported a negative outcome of their experiments, while Saxton and Yuasa recorded occasional or extensive lipid deposits in the aortic intima. The reason for these divergent results in rats is not apparent from the published data. In feeding mice with various kinds of fats Domagk obtained in addition to amyloid and leukemoid changes in the liver and the spleen fatty degenerations and calcifications of the walls of arteries. Rabl recorded similar results as to arterial lipoidosis and calcinosis in mice fed a cholesterol-containing diet adjusted by the addition of phosphoric acid, ammonium chloride, potassium sulfate, calcium phosphate and sodium acetate to an alkaline or an acid reaction. While Lowenthal was unsuccessful in eliciting atheromatous reactions in normal mice fed a cholesterol-containing normal diet, he succeeded in this respect in castrated mice and in mice fed a protein-cholesterol diet. Corwin mentioned briefly that attempts to produce alimentary atheromatosis in foxes were unsuccessful.



Experimental cholesterol atheromatosis affecting the aorta and the brachiocephalic, coronary, renal, celiac, iliac and femoral arteries was relatively readily and consistently obtained in young chickens by several workers (Uchiyama, Wolkoff, Dauber and Katz). Chickens behave like mammals in regard to their cholesterol metabolism, according to Sperry and Stoyanoff. The cholesterol content of the serum was markedly elevated in chickens fed cholesterol (430 to 2,148 mg per hundred cubic centimeters) (Dauber and Katz). The ratio of free cholesterol to esters remained within normal limits. The resulting intimal lesion consisted of extracellular and intracellular lipoidal deposits associated with fibroblastic and elastic fibrillar proliferations. Calcifications and ossifications within the foci were not rare. The intimal cushions sometimes encroached markedly on the lumens of large and small arteries, occasionally causing almost complete obliteration. In such severe lesions intimal foam cell proliferations invaded the media, which was often calcified. In addition to lipoidotic changes in various parenchymatous organs, the testes were atrophic, similar to those seen in cholesterol-fed pigeons with atheromatous arterial changes (Kawamura, Yamaguchi). The vascular lesions showed a topographic distribution similar to that of the juvenile lipid spots seen in man. Dauber and Katz suggested that the difference in the localization of the atheromatous reactions in man and chickens is probably attributable to the fact that different functional and anatomic strains are exerted on various parts of the aorta of these two species. These investigators noted that the pulmonary arteries and veins were free from atheromatous lesions, while the vasa vasorum of the aorta were filled with foam cells. This observation led Dauber and Katz to believe that the migratory foam cells clogging the vasa vasorum initiate the degenerative changes (necrosis, deposition of cholesterol, fibrosis, calcification) in the vascular walls.

For the proper evaluation of these observations it is necessary to point out that Sperry and Stoyanoff found markedly increased serum cholesterol in some of their chickens kept on a normal diet, while Kraus noted that chickens start to show intimal lipoidal deposits in the large arteries from their second year of life. These lipoidal foci are located mainly in the ascending and abdominal portions of the aorta and involve both the intima and the media. Kraus concluded from this evidence that reports of experimental production of atheromatosis in chickens are of uncertain interpretation.

Hesse and Wolkoff, who reported the occurrence of severe and generalized atheromatosis and calcinosis of the arterial tree in 3 parrots kept in captivity for many years and fed a diet of nuts and eggs, considered these manifestations as results of alimentary cholesterosis. It may be mentioned, however, that Cook failed to observe such changes in a parrot receiving a diet fortified with cholesterol and that similar arterial changes are commonly seen in old parrots (Nieberle, Pallaske, Wolkoff, Fox).

Among the herbivorous animals, monkeys, goats, guinea pigs and rabbits have been used for the experimental production of alimentary cholesterol atheromatosis. All attempts to elicit such lesions in monkeys failed (Duff, Corwin). Chalataw fed 3 goats egg yolks for two and a half to five months and obtained a threefold increase of the serum cholesterol in a pregnant female goat and in a young male goat, while the blood cholesterol level of an old male goat remained unchanged. The aorta of the young male goat showed many intimal intracellular lipoidal deposits located especially around the orifices of the aortic branches. The aortas of the older animals were without any significant changes. Chalataw fed guinea pigs egg yolk for a relatively short time without obtaining atheromatous arterial reactions. Anitschkow, who repeated these experiments and extended them for up to one hundred and eighty-three days, produced typical atheromatous intimal responses in the aorta. Pseudoxanthoma cells located between the endothelium and the internal elastic membrane had taken up lipid material primarily deposited in the interstices of the intima. Similar results in guinea pigs were recorded by Bailey, Cook and Schonheimer. Cook and McCullagh, who fed guinea pigs cholesterol in arachis oil for up to two hundred and eleven days and obtained marked hypercholesteremia, found, on the other hand, only minor intimal lipid deposits in the aorta and the large vessels.

The great majority of experiments on alimentary cholesterol atheromatosis were performed on rabbits. In the original experiment of Ignatowski, which started the experimentation on alimentary cholesterol atheromatosis, rabbits received a diet of milk and egg yolk, which is rich in cholesterol. After the demonstration of cholesterol as the active principle in this diet subsequent investigators followed several lines of dietary management in their experimental studies on rabbits. One group of workers (von Leesum, Warischew, Anderson, Newburgh and Squier, Newburgh and Clarkson, Knack,

Meeker and Kesten, Stuckey, Fahr, Duff, Starokadomsky, Saltykow, Steinbiss, Clarkson and Newburgh) continued to feed a diet containing cholesterol as a part of a proteimic base, such as egg yolk, milk, brain, powdered liver and meat. The majority of workers used cholesterol dissolved in an oily medium (sunflower oil, olive oil, linseed oil, arachis oil, cottonseed oil, sodium oleate), which was either mixed with the basal diet or given by stomach tube (Versé, Katz, Sanders, Megibow and Carlen, Leary, Liebig, Anitschkow, Aylward and Stott, Rosenthal, Bollman and Flock, Matsunami, Friedberg and Hurwitz, Duff, Schmidtman, Schonheimer, Hirsch and Weinhouse, Weinhouse and Hirsch, Wolkoff, Zinserling). A few investigators (Diez, Shapiro, Chuma, Nisi) fed cholesterol in the forms of hydrous wool fat, whereas only the exceptional student added dry cholesterol to the ordinary diet (Wacker and Hueck, Scarff, Knack). Atherosclerosis was successfully produced by all these dietary procedures, but relatively least readily by the last-mentioned method.

The alleged production of intimal lipoidal deposits following the administration of lecithin (Remesow, Seemann) has to be charged to the cholesterol usually present in preparations of lecithin of animalic origin (Schonheimer). Lecithin prepared from soybeans apparently has no atherosclerogenic properties. Wesselkin's results with lecithin were negative. The feeding of vegetable protein (soybeans, gluten flour) (Freyberg, Stuckey) or of defatted liver (Kon) or of various kinds of oils and fats free from cholesterol (Versé, Duff) was equally ineffective. Diez, on the other hand, asserted that the feeding of spermaceti, which does not contain any cholesterol, caused hypercholesteremia. Raab stated that whenever steps have been taken to remove the cholesterol from the proteimic matter the feeding of this material fails to elicit atheromatous lesions.

It must be mentioned in this connection that the feeding of a protein diet free or almost free from cholesterol to rabbits has repeatedly resulted in vascular changes of a sclerotic-calcinotic or sclerotic-lipoidotic type. Steinbiss reported that in rabbits kept on a diet of powdered liver or testis there developed, simultaneously with osteoporosis, only degenerative changes in the muscular and elastic elements of the aortic media, associated with fibrosis and calcinosis. Similar lesions were sometimes seen in the small arteries of the kidney, the liver and the spleen. Nuzum, Osborne and Sansum repeated the experiments of Steinbiss and observed in their rabbits the development of hypertension and arteriosclerotic

lesions consisting of swelling and calcification of the intimal ground substance and hyalinization and calcification of the elastic fibrils and of the muscle cells of the media. Nuzum, Seegal, Garland and Osborne observed during subsequent experiments performed on rabbits which received for two years a pure oat diet and in which hypertension developed transitory swelling of the intimal ground substance followed by deposition of cholesterol and hyalinization and fibrosis of the intima. Through cyclic repetition of these processes there occurred a layered thickening of the intima with lipoidal-hyaline deposits. In both the liver-fed and the oat-fed rabbits acidosis developed as a result of the diet, and Nuzum and his co-workers attributed to it the production of the hypertensive and atherosclerotic reactions. They considered a dietary cholesterol intake as unessential for the production of atheromatous lesions, on the basis of these observations. Meeker and Kesten recently took up again the feeding of rabbits with a cholesterol-free protein (defatted casein), added to the regular diet, and observed in the rabbits thus treated the development of mild to moderate hypercholesteremia and of arteriosclerotic changes in the aorta and the coronary vessels. When the casein was replaced in the diet by soybeans, the production of atherosclerotic lesions was prevented by the iodine normally contained in soybeans.

In this connection reference may be made to experiments made by Newburgh and Clarkson and by Anderson. These experimenters fed rabbits a diet 50 per cent of which was powdered lean beef meat. The rabbits obtained thereby a total daily cholesterol intake of 28 mg, and typical atheromatous lesions developed in the aorta in addition to intimal and medial calcifications. Newburgh and Clarkson were inclined to disregard the possible role which the small amount of ingested cholesterol may have played in the production of the vascular responses and expressed the belief that these reactions were the result of the protein diet. These observations suggest the possibility that in rabbits certain types of protein diet may elicit under proper experimental conditions of unknown nature hypertensive, hypercholesteremic and atheromatous or atherosclerotic or arteriosclerotic-calcinotic reactions ensuing from disturbances of the calcium and cholesterol metabolism.

These observations are of special interest as certain workers (Schmidtman and Huttich, Schmidtman, Westphal, Deicke, Fahr, Matusita, von Leersum, and Schonheimer) have asserted that the feeding of cholesterol, egg yolk,

hydrous wool fat or liver results in the development of hypertension, which is causally connected with the hypercholesteremia produced, since renal changes are absent. Schmidtmann proposed that the hypertension is attributable to a sensitizing effect exerted by the cholesterol on epinephrine. The hypertensive reactions paralleled the atheromatous vascular changes, according to Schmidtmann. These observations, however, were not confirmed by others (Tregubow, Shapiro and Seecof, Thollde, Shapiro, Shepard and Seecof, Katz, Sanders, Megibow and Carlen). The cardiac hypertrophy noted in cholesterolized rabbits by Katz, Sanders, Megibow and Carlen and by Friedberg and Hurwitz could not be traced to systemic hypertension but was attributed to the myocardial ischemia caused by the atheromatosis of the coronary arteries, which, on the other hand, did not elicit abnormal electrocardiographic reactions (Nyboer, Bruger and Rabson). These experimental data indicate in connection with the clinical experience that hypercholesteremia and hypertension are not causally interrelated.

The increase in the serum cholesterol content of the cholesterolized rabbits may be considerable (up to twenty times the normal amount) and is not accompanied by any consistent changes in the ratio of free to esterified cholesterol. While this reaction is accompanied by elevation of the levels of the other lipoids and lipids of the serum (Weinhouse and Hirsch, Wacker and Hueck, Bollman and Flock, Page and Bernhard), the cholesterol increase is disproportionally higher than that of any other of the fat substances. The rise in serum cholesterol during the first two months under this dietary management is a gradual and progressive one. After two to three months the movement levels off and is followed by a drop in spite of continued oral administration of cholesterol. The serum cholesterol level finally becomes stationary at a point several times the normal value. It is noteworthy that this drop cannot be prevented by increasing the dose of cholesterol ingested but can be slowed by increasing the amount of oil given (Verse, Weinhouse and Hirsch, Reineck, Kirchgessner, Rohrschneider). There seems to develop first a balance between absorption, on one hand, and excretion and storage, on the other. After all possible depots for lipid storage in the organism are filled to capacity there follows apparently a metabolic adaptation of the body to the increased intake of cholesterol, resulting in either more rapid excretion of the cholesterol or decreased absorption of it from the intestine.

It is noteworthy that the type of oil used as a solvent influences the speed of absorption of cholesterol and of the development of hypercholesteremia. Linseed oil seems to be most effective in this respect (Versé).

The deposition of cholesterol in the arterial walls is usually preceded by considerable storage of lipoids in various organs (liver, adrenal glands, kidneys, spleen, lungs) and in the reticuloendothelial system when the usual method of feeding large daily doses of cholesterol is used. The chronic effects of this thesaurosis elicit cirrhotic changes in the liver (Versé, Broun and Muehler). Lipoid deposits in the corneas (arcus senilis) of cholesterolized rabbits are frequent (von Poppen, Rohrschneider, Versé, Kolen, Chuma, Schonheimer, Nakanonim, Nakanonim and Koboschi, Kawamura, Wada, and others). These corneal changes recede partly after cessation of the oral ingestion of cholesterol. Marked differences exist in the degrees to which the various lipoids and lipids are stored in the different organs (Aylward and Stott). The organic retention of glycerides appears to be independent of that of cholesterol and to be influenced by the choline, protein and oil contents of the diet.

Experiments of Anitschkow, of Chuma and of Zinserling and Krimitzky have demonstrated that the development of organic lipoidosis and of high hypercholesteremia is not prerequisite and essential to the development of alimentary atherosclerosis in rabbits. When Anitschkow fed rabbits with small amounts of diluted milk and egg yolks and when Chuma and Zinserling and Krimitzky administered small quantities of hydrous wool fat to rabbits over periods of two years, they noted isolated and mild atheromatous reactions in the aorta in the absence of storage phenomena in the parenchymatous organs and in the presence of only minor hypercholesteremia. The causative and hematic conditions present in these experiments resemble closely those observed in many instances of human atherosclerosis. Anitschkow showed, moreover, that a short and intensive course of oral treatment with cholesterol is sufficient to elicit atheromatous lesions, which are formed in the presence of minor organic lipoidosis and which persist and are newly formed for several months after the arrest of treatment and when the serum cholesterol level has returned to normal. The absence of hypercholesteremia is thus not proof that such a disturbance did not furnish the causal background of atherosclerosis.

Atheromatous deposits in the arteries appear first and are most marked in the ascending por-

tion of the aorta and then around the orifices of the intercostal arteries. Later they extend to the abdominal portion and to the larger arteries—the carotid, subclavian, iliac, renal, splenic, intestinal, renal and coronary arteries (Duff, Anitschkow, Hurwitz and Friedberg, Wolkoff, Schmidtman, Scarff, Katz, Sanders, Megibow and Carlen, Versé, Cook and McCullagh, Leary). The large and small branches of the pulmonary artery are frequently involved, while with severe nodular xanthomatous atheromatosis the veins also show lesions. Schonheimer observed endothelial and subendothelial deposition of lipid in the intima of the inferior vena cava and in the portal vein, which was similar to that seen occasionally in man with high hypercholesteremia (Benda).

The atheromatous formations start with the appearance of lipoidal matter in the subendothelial ground substance and in foam cells of this region or with foamy swelling of the endothelial cells. The subendothelial foam cells are in part derivatives of the proliferated xanthomatous endothelial cells, but mainly they evidently originate from histiocytic cells, especially in those cases in which they appear beneath an apparently intact endothelial lining. Anitschkow and also Leary, however, insist that the foam cells are exclusively of histiocytic or reticuloendothelial origin. The internal elastic membrane may show fraying and proliferation and in severe lesions is penetrated by foam cells invading the media. In advanced changes there may be foam cell transformation of the endothelial lining of the vasa vasorum as well as accumulations of foam cells in the spaces around these vessels. Medial calcifications beneath the foam cell intimal cushion are occasionally seen (Danisch, Hueper).

Early regressive changes in these lesions were observed by Stuckey and by Krylow during the first six months after the cessation of cholesterol feeding. Verse reported fibromuscular transformation of the plaques with central calcification in a rabbit which survived the dietary treatment by four hundred and twenty-five days. Wada recorded similar reactions in rabbits after hydrous wool fat had been fed. Anitschkow, who studied the aortas of rabbits one hundred and one to eight hundred and fifteen days after the discontinuation of the cholesterol management, found the first signs of regressive changes in the atheromatous lesions in the ascending and upper thoracic portions of the aorta, such reactions did not appear in the atheromas of the abdominal portion of the aorta until much later. During the regressive changes the lipid first disappears from the foam cells and re-

mains only in the periphery of the plaque, while the center is replaced by fibrous tissue. Collagenous and elastic fibrils develop between the foam cells. The fat released from the disintegrating foam cells separates into large coalescing globules of neutral fat and cholesterol crystals. The neutral fat is resorbed, while the cholesterol crystals remain. Calcium granules then appear in the deeper parts of the plaques, and the fibrosis increases (Anitschkow, Duff). New formation of capillaries is only rarely seen in the atheromas (Anitschkow). Saltykow observed similar regressive changes in rabbits maintained continuously on a milk diet. The development of aneurysms of the aorta in the region of the arch on the basis of these regressive intimal and medial changes was reported by Liebig, by Wesselkin and by Leary and Weiss. In the case reported by Leary and Weiss a dissecting aneurysm was found similar to those frequently observed in the rabbit's aorta after the injection of epinephrine hydrochloride (Fischer, Erb, Kulbs, Ziegler, Kaiserling, Schirokogoroff).

Numerous experiments have been performed in attempts either to accentuate or to accelerate the development of dietary cholesterol atheromatosis in rabbits or to impair or to suppress this process. Ssolowjew succeeded in aggravating the local deposition of cholesterol in exteriorized parts of carotid arteries, which were placed in skin sleeves. These arteries were traumatized when the rabbits threw their heads violently backward during daily attempts to introduce a stomach tube. These abrupt movements of the neck produced tears in the elastic membranes. The defects thus produced were filled in by lipid-containing foam cells. While Schmidtman recorded aggravation of the vascular lesions in cholesterolized rabbits through administration of vitamin D, Harrison observed that vitamin D sclerosis in rabbits rendered the affected parts relatively immobile and that this immobility seemed to make the sclerosed foci less susceptible to the subsequent development of atheromatous lesions. Meeker and Kesten reported that a diet high in soybean flour diminished the incidence and the degree of experimental atheromatosis, while a diet rich in defatted casein augmented them. The attempts of Jobling and Meeker to accelerate or to increase the development of cholesterol lesions in the aortas of rabbits failed when the following procedures were employed: Intravenous injection of streptococcus toxin, feeding of ammonium hydroxide, production of artificial fever, intravenous injection of peptone, production of anaphylactic shock and intravenous injection of uric acid. These steps were taken

so as to elicit one of two apparently primary changes in the intima important in the development of experimental and human atherosclerosis (1) a change in the permeability of the wall of the vessel in the neighborhood of the internal elastic membrane which prevents the normal passage of large molecules, such as proteins and lipids, from the intima into the media without interfering with the passage of water and crystalloids, (2) an increase in the permeability of the intima without other injury to the vessel wall. In both instances it was thought that there may occur a deposition of relatively large amounts of lipids and of coagulated and finally hyalinized protein. Similar experiments of Thiersch, who tried to enhance atheromatosis by producing bacterial allergies, likewise failed.

Shapiro noted that thyroidectomized, splenectomized or gonadectomized rabbits exhibited increased susceptibility to cholesterol atheromatosis. This susceptibility was especially marked in thyroidectomized animals. Mochlig's attempts to attain this goal in cholesterolized rabbits by repeated injections of a solution of the posterior lobe of the pituitary gland were successful. Injections of the solution alone proved to be ineffective. A similar aggravating effect was exerted on the atheromatosis by the thyrotropic factor of the pituitary gland (Bruger and Fitz, 1931).

Various means have been employed in the many attempts made to prevent or to cure the cholesterol atheromatosis of rabbits. Alcohol recommended by Leary as an antidote against cholesterol atheromatosis in man, was tried by Eberhard in cholesterolized rabbits. It was found that if the cholesterol was dissolved in 20 per cent alcohol, the absorption of the cholesterol from the intestine was accelerated and the rise of the blood cholesterol level was more rapid and higher than when cholesterol was added dry to the basal diet. While there were no gross differences in the appearance of the atheromatous aortas of the two groups, the lesions in the alcohol series exhibited smaller intimal cushions which extended less often into the media. The significance and the finality of these observations, however, were regarded by Eberhard as doubtful. Schaffir fed egg yolk and alcohol to rabbits and observed increased aortic atheromatosis and medial calcifications. Thiersch reported similar results in rabbits equally treated with cholesterol and alcohol. Chaika noted that lactic acid and oleic acid when fed to rats along with cholesterol increased the resulting hypercholesterolemia but prevented deposition of fat in the aorta. These acids also intensified the uptake of lipids by the cells of the reticuloendothelial system. After

Buigi had contended that chlorophyll exerted a favorable effect on arteriosclerosis and hypertension, Blumer, Gordonoff and Reznikoff and Gordonoff fed rabbits cholesterol in oil and a preparation of chlorophyll and observed only minor fibrotic thickenings in the aortic intima and absence of lipemia and lipoidosis of the Kupffer cells. Malisoff and Hueper claimed that the administration of potassium thiocyanate reduced the severity and the incidence of atherosclerosis in thyroidectomized rabbits fed cholesterol. The mechanism of this protective action is according to Malisoff, not clear, as it is uncertain whether the thiocyanate heightens the dispersion of the cholesterol colloids in the blood and the tissues or changes the permeability of the vascular wall (Westphal), as thiocyanates are colloidochemical antagonists of cholesterol (Guttmann).

Attempts of Hesse to influence favorably the cholesterol atheromatosis of rabbits by the administration of an ethyl ester of ricinoleic acid and of its silicic acid adduct were unsuccessful. Equally unsuccessful were the efforts of Flexner, Bruger and Wright to prevent the development of hypercholesterolemia and atheromatosis in cholesterolized rabbits by the injection of massive doses of ascorbic acid and thiamine singly or combined, as well as those of Thiersch in connection with the administration of dehydrocholic acid.

While the experimental results of Kesten and Silbowitz suggested that the administration of soybean lecithin or of choline in an amount equivalent to that in lecithin mitigated the development of cholesterol atheromatosis in rabbits, the studies of Steiner, of Himsworth and of Baumann and Rusch showed that the administration of even large doses of choline had no influence on the cholesterolemia and atheromatosis of cholesterolized rabbits. Steiner reported however, as a suggestive finding that choline might aid in the mobilization and resorption of lipoids from atheromatous lesions produced in the aorta. Although Huber, Brown and Casey reported that lipocaine prevented the development of hypercholesterolemia and atheromatosis in cholesterolized rabbits, subsequent investigations (Vermeulen, Allen, Clark, Julian and Dragstedt, Vermeulen, Dragstedt, Clark, Julian and Allen, Dragstedt, von Prohaska, Clark and Julian) did not confirm this claim. Wright contended, on the other hand, that the administration of a deproteinized pancreatic extract to cholesterolized rabbits retarded the development of atheromatosis and caused a striking transitory lowering of the blood cholesterol level. Ludden, Bruger and Wright showed that estradiol dipropionate and testosterone propionate had no in-

fluence on the cholesterol content of the blood and the aorta when given over a period of one hundred days. However, when female rabbits fed cholesterol were treated with either of these substances they showed inhibition of the hypercholesteremia associated with almost complete absence of cholesterol deposits in the aorta. No such results were seen in male rabbits identically treated. Gonadectomy did not alter the response of normal female rabbits, but the protective action of these substances toward arteriosclerosis was abolished after castration. These observations are important, as atherosclerosis and medial calcinosis are in general less frequent in women than in men. The frequency of coronary arteriosclerosis is thus four and nine-tenths times as great in men as in women (Levy and Boas). Similar percental conditions exist in regard to thromboangitis obliterans, suggesting a protective action related to estriogen.

The majority of experimental investigators in this field have used either iodides, which in many respects resemble cyanides in their biologic and physicochemical action (Westphal), or thyroid preparations. Iodides have been employed for many years in the treatment of arteriosclerosis and hypertension, mainly on an empiric basis. Numerous explanations have been advanced for the alleged favorable results obtained clinically in patients. Guggenheimer and Fisher stated that iodine has a vasodilative effect (See and Lapique, Krause, Bogolepoff, Rose, Huchard, Eloy, Bloom, Kochmann, Liebe). This assertion was contradicted (Boehm and Berg, Barkan and Prikk, Orth, Eppinger and Hess, Freund and König). Some have contended that iodides decrease the volume of erythrocytes and thus lower the blood viscosity, thereby relieving hypertension (Romberg, Muller and Inada, Deutsch and Frowein). This claim also was disputed (Determann and Broking, Alwall, Buchholtz, Boruttau). Adam even found an increase in blood viscosity after iodide medication. Others attributed the beneficial effect of iodide on atherosclerosis to an increase in pulse volume and thereby an improvement in circulation (Lehndorff) or to complex endocrine and metabolic reactions (Hildebrandt, Hesse, Wegelin, Wada, Mansfeld, Baikan) or to a direct action on the atheromatous material (Ungar, Loeb) or to an effect on vegetative nervous centers (Schittenhelm and Eisler, Abelin, Sturm and Veil) or to an inhibition of the accumulation of cholesterol deposits in the arterial walls (Damrau). Schottmuller, as well as Masing, on the other hand, did not see any favorable effect from iodides in arteriosclerosis, while Turner and Steiner observed either no effect on the serum cholesterol level of patients

with various diseases or exceptionally even an increase after prolonged introduction of iodides.

These contradictory observations in man, which are in part evidently due to the fact that arteriosclerotic patients were subjected to medication with iodine without regard to etiology or type, contrast sharply with those made on rabbits treated for dietary cholesterol atheromatosis either with an inorganic iodide (potassium iodide or sodium iodide) or with various organic iodides (Liebig, Meeker, Kesten and Jobling, Bruger and Fitz, Murata and Kataoka, Seel and Cleuzberg, Friedland, Page and Bernhard, Turner and Khayat, Strauss, Turner and Bidwell, Masson, Page, Turner, Ungar, Thiersch). All investigators found that iodides given together with cholesterol prevent or mitigate the development of cholesterol atheromatosis. Organic iodides, especially compounds of fatty acids, were apparently more effective than inorganic compounds because they remain in the blood longer than the inorganic iodides (Broking). Their effect is apparently not dependent on a reduction in the serum cholesterol level, although this reaction is commonly seen after the administration of iodides. Page and Bernhard, who used a diiodide of ricinoleic acid, found in their iodide-treated series higher lipemia than that present in the controls which received cholesterol only. While fatty acid-iodine compounds retain their effectiveness in thyroidectomized rabbits (Ungar), the effect of inorganic iodides depends on the presence of the thyroid gland, for their protective action is abolished after the removal of this gland (Turner and Khayat). The complexity of these reactions is indicated by the fact that the favorable effect of iodides is not a simple metabolic oxidation effect exerted through the thyroid gland or a hypocholesteremic effect but is probably connected with a stabilizing action on the equilibrium of the plasmatic colloids. Inasmuch as iodide medication has no influence on lesions once established, this type of therapy is of purely preventive and not therapeutic character (Thiersch).

A similar protective action in rabbits is demonstrable when thyroid preparations are given together with cholesterol (Zon, Murata and Kataoka, Friedland, von Baló, Menne, Beeman and Labby). In cholesterolized rabbits there was a reduction or an absence of hypercholesteremia as well as an absence or a reduction of atheromatous manifestations. In contrast to prolonged iodide medication, which finally failed to check the development of a hypercholesteremia and sometimes even accentuated this reaction, the administration of thyroid preparations maintained the hypocholesteremic effect unchanged.



(Turner and Steiner) The inconstancy of the iodides in this respect may be attributable to the fact that prolonged iodine medication may elicit a hypothyroidotic, myxedematous state following a primary hyperfunctional response.

Leary and Raab recommended as a practical measure for the control of atherosclerosis in man a restriction of the dietary intake of cholesterol through a lessened consumption of butter, egg yolks and meat. This stand appears to be well taken so far as it concerns persons whose consumption of these substances is highly excessive and persons with disturbances of the fat metabolism (diabetes mellitus, obesity, essential xanthomatosis, psoriasis).

Many investigators maintained that the experimental alimentary cholesterol atheromatosis of rabbits differs in several fundamental aspects from the human atherosclerosis and that therefore no reliable conclusions can be drawn from the observations made on rabbits as to the causative mechanism and the etiologic factors of this condition in man (Klotz, Dufl, Hirsch and Weinhouse). It is often maintained that the cholesterol atheromatosis of rabbits resembles the lipid spots in man or the xanthomatous vascular lesions seen in essential xanthomatosis but not typical atherosclerosis. It is pointed out that the atheromatosis of rabbits occurs only in association with and as a sequela of generalized lipoidosis, that it is obtained only when rabbits are fed an abnormal diet, that it differs in the topographic distribution of its lesions from the human variety, that the histologic structure of the atheroma varies from that of the human type, that the same measures fail to produce similar lesions in carnivorous animals, that in rabbits the cerebral arteries are spared, while they are often affected in man, and that the atheromas of rabbits do not ulcerate, with development of thrombotic deposits.

Although it must be conceded that the lipid metabolism of the rabbit differs in certain respects from that of man, this does not detract from the fact that in both species disturbances of the cholesterol content of the plasma are associated with atheromatous arterial lesions of essentially the same type. Hypercholesteremia and instability of the plasmatic colloidal lipoidal solution represent the common denominator for the development of the organic lipoidoses and the vascular atheromatosis seen in the acute alimentary form of cholesterosis of the rabbit and the accelerated types of metabolic cholesterol disturbances accompanying essential xanthomatosis, diabetes mellitus and hypothyroidism. The hematic and vascular reactions seen in rabbits fed small amounts of cholesterol over long periods are not

unlike those observed in many instances of the ordinary type of human atherosclerosis. It is relatively immaterial as far as the immediate causative mechanism in man is concerned whether or not an excessive alimentary intake of cholesterol plays a significant role in the production of the atherosclerotic lesions, as there are many other causes for the imbalance in the plasmatic colloidal lipoidal solution. The evidence available in this respect in man together with the fact that alimentary factors are apparently involved in the development of atherosclerosis in other omnivorous animals, makes such a connection not unlikely. It can scarcely be maintained that at the present stage of knowledge of human nutrition any exact statements can be made as to how normal or abnormal the diet of natural and processed foodstuffs of the average human being is, especially if consideration is given to the differences in constitution and environment, and as to what long range effect it exerts on the plasmatic lipoidal equilibrium and the status of the vascular system.

The differences in the topographic distribution of the atheromatous lesions in man and rabbits are evidently in part due to differences in localizing static factors. This conclusion is supported by recent observations made by Wilens, who was able to produce a shift of the atheromatous manifestations from the ascending part of the aorta into the abdominal part by keeping cholesterolized rabbits for five hours daily in an erect position, imitating thereby the static conditions present in man. It is likely, moreover, that differences in the course and in the structure of the various arteries of man and rabbits exert a definite influence in this respect, as is apparent from observations made in man and rabbits as well as in other species. Moschcowitz laid main emphasis on the local fixation and the external resistance of the vascular wall, which control the expansive mobility of the vessel, as the cause of the localization of atherosclerotic lesions under the influence of increased intravascular hydrostatic pressure. In support of his thesis he pointed to the following observations: 1 The earliest patches of aortic sclerosis are at or near the origin of intercostal vessels which fix the posterior wall of the aorta. 2 The abdominal part of the aorta, which is fixed against the spine, is more involved than the thoracic. 3 Dural vessels are most markedly involved when lying in bone. 4 The anterior aspect of the aorta is less involved than the posterior, as the latter is fixed against the spine. 5 Radial arteries show the most marked sclerosis where they lie directly against the radius. 7 Aortic patches appear ear-



liest in places corresponding to upper and lower borders of bodies of vertebrae 8 Pulmonary arteries lying against rigid bronchial cartilage are most involved 9 The left coronary artery, embedded in firmer muscle, is more affected than the right one

Aschoff and many others asserted that the areas around the orifices of intercostal arteries and at arterial bifurcations are exposed to increased mechanical strain and thus are subject to degenerative changes which favor the infiltration of plasma and the precipitation of cholesterol Harrison maintained that the movements of a vessel determine the localization of cholesterol lesions in animal as well as probably also in human arterial disease, since local immobility appears to render the area in which it is present less susceptible to subsequent atheromatous lesions Oberndorfer found in man no atheromatosis in arteries overlying joints (popliteal space), though these parts of vessels are often in motion

Ranke pointed out that lumbar lordosis developing during life as a result of erect posture influences the blood current in the aorta and may explain the higher incidence of atherosclerosis in the abdominal part of the aorta Atherosclerosis, according to this investigator, involves earliest and most extensively those vascular regions in which the compensation to the hematic push-tension of the wall is disturbed or lowered for some reason (as in the concave parts of curves, at bifurcations) and where the range of compensation is lowered The concave part of the aortic arch is much more stretched by the dislocation of the heart when a full stomach is present than the convex side Distention in width means a shortening pull in length These influences interfere with the nutritive current in the wall by impairing the outflow of tissue lymph, and thus edema develops which causes tension of the fibrils, which in turn impairs the attachment of the intima to the media At such spots atherosclerosis develops The predominating location of atheromatosis in the abdominal part of the aorta is explained by the fact that only here a sufficient longitudinal shift of the aorta occurs causing diffuse changes, while the nodular lesions are the result of abdominal pressure exerted on the abdominal portion of the aorta by the abdominal wall The location of atheromas on the posterior aspect of the aorta is attributable to the circumstance that the aorta is not extendable in this region, as it is fixed to the spine (Ranke)

Albrecht noted that the internal carotid artery embedded in the petrous bone shows more fatty degeneration and calcification than the common carotid artery or the axillary artery Smetana

attributed the early occurrence of atheromatosis in the aortic bulb to the action of a functional strain surpassing that of other parts of the vessel, while the distribution of the vasa vasorum does not play a role in this respect

Differences in the structure of the arterial walls of man and rabbits, particularly in the thickness of the intima and in the amount and the distribution of the elastic tissue, probably exert an additional modifying influence on the distribution of the atheromatous lesions The importance of this factor is evident from the following statements Duff proposed that cholesterol is precipitated at sites where the vascular wall is altered, i e., where primary focal destruction of the muscle fibers or edematous swelling of the subendothelial ground substance occurs On the basis of observations made during experiments in which typan blue was injected into rabbits, he contended that the permeability of the intima of the aorta of the rabbit to typan blue is uniform throughout and that the local accumulations of the dye in the aortic wall are related to the distribution of the vasa vasorum from which the dye leaks out into the media The intensification of the dye around the orifices of the intercostal vessels is attributed to increased tissue lymph flow through these regions rather than to slowing of it The same causative mechanism, it is held, accounts for the accumulation and precipitation of cholesterol in these areas

Rosenthal, on the other hand, favored the concept that slowing of the tissue lymph stream in the intima with the elastic barrier acting as a hindrance to permeation of the media by lipoids is responsible for the accumulation of the lipoids in the intimal tissues, but he did not provide a sufficient reason for their precipitation A similar conception was proposed by Anitschkow, who stated that the occurrence of cholesterol deposits depends on general conditions (status of the blood and the nutritive fluid permeating the vascular walls) and on local conditions determining the exact site of the deposits (local injury of the wall caused by change in the ground substance) The proliferative responses accompanying this process he considered as secondary Pekelharing noted that the rhythmic changes in the intravascular pressure with the pulse exert the smallest pressure where the vascular wall yields most The local loss of elasticity thus furnishes the focus for intimal proliferation following local vascular dilatation A similar concept is propounded by Dormanns and Emminger, who claimed that the marked appearance of atherosclerosis in the ascending and thoracic parts of the aorta in the presence of syphilitic

aortitis is attributable to the formation of local aortic dilatations due to the destruction of elastic tissue

Leary stated that the first lesions occur at sites of special mechanical stress which causes, however, no injury to the intima. With the closing of the aortic cusps the ascending part of the aorta is subjected momentarily to greater stress than any other part of the arterial system. As the proximal portions of the coronary arteries share in this stress, this mechanism accounts for the selective frequency of sclerosis in these parts of the coronary arteries. A static aspect of atherosclerosis and medionecrosis of the arteries in the lower extremities receives some support from the observations of Man and Peters and of Keys and Butt, who found that on standing there is an increase in the lipid and protein contents of the blood of these vessels as watery components leak through the vascular walls under the influence of increased static pressure causing increased permeability of the vascular walls. Beneke pointed out that the blood flow is normally not uniform but whirl formation occurs especially at curves, orifices of branches and bifurcations. The increased stress entailed thereby provides the cause for the localization of atheromas.

While the factual observations concerning the distribution of atheromatous lesions at various special points of the arterial tree are correct, none of the numerous reasons offered in explanation of the findings appears to be satisfactory from a colloidochemical standpoint, and many of the arguments advanced disregard entirely the colloidal aspect of the production of the lesions. If slow blood flow and stagnation of tissue fluid represent an important feature of the accumulation and precipitation of lipoids in the vascular wall, it must be expected that not the arteries but the veins would be the location of atheromatosis. In fact, atheromatosis is found most marked in those vessels in which a fast blood flow prevails. If the distribution and the plugging of the vasa vasorum with lipid cells are of significance, again the venous walls should be the most frequent site of atheromatosis, as the veins possess a well developed network of vasa vasorum which extends into the intima and have a slow blood flow favoring the arrest of lipophages on the vascular walls and then invasion of the vasa vasorum. The actual distribution of the atheromatous reactions is fundamentally different.

If the preferred sites of atheromatous deposits in the arterial tree are analyzed from a hemody-

namic point of view, it becomes apparent that they represent spots where the uniform blood flow is acutely disturbed, i. e., where turbulence of the blood occurs. It is obvious that at the base of the aorta is an area in which the blood is exposed to frequent and violent vibration due to the recoil of the blood during the period of diastole as well as during the systolic impact of the cardiac blood on the aortic blood column. The resulting whirl formation, doubtless, is accentuated whenever the aortic wall is studded with small retractions which break the marginal blood current and which are formed as the result of syphilitic scarring of the media. The blood in the proximal parts of the coronary arteries is subjected to a similar mechanism when during systole the intramyocardial branches are obliterated by the contracting myocardial tissue and the coronary circulation comes practically to a temporary standstill. The blood is then pressed into the extramyocardial parts of the coronary vessels and especially into their proximal portions, setting up there violent whirl formations. Any local dilatation of the vascular wall whether caused anatomically by abnormal focal changes in the wall or resulting functionally from a one-sided circumscribed fixation or external resistance, exerts a similar effect on the blood current. Natural or pathologic narrows of vessels, such as passages through bone or the presence of orifices of branches, bifurcations and curves, also increase the turbulency of the blood stream in circumscribed parts of the arterial tree.

Hellwig recently pointed out that atheromatosis of the mitral valve is apparently due to the fact that the colloiddally dispersed lipoids are precipitated on the ventricular side of the mitral leaflet because the stability of the plasmatic cholesterol solution is disturbed by the vigorous systolic percussion of the mitral leaflets, with precipitation of the lipoids. The phenomenon is comparable as to mechanism to the lipid flocculation elicited by shaking in the Kahn test. Similar changes were reported by Hueper in connection with his experiments with polyvinyl alcohol, which is precipitated out of solution on shaking. It seems reasonable to assume that such vibratory disturbances of the plasma are responsible for the distribution of the atheromatous lesions in the vascular tree at the aforementioned sites.

The deposition of the precipitated lipoids depends in part on such local factors and in part on static conditions, as the precipitated material tends to settle in the dependent portions, i. e. in the abdominal part of the aorta of the full

grown person. This influence will be much less pronounced or even absent in the young person in whom such static conditions are either non-existent or only mildly active. The experiments of Wilens on cholesterolized rabbits kept in an erect position support the soundness of this reasoning.

It is not unlikely that the different physico-chemical conditions of the arterial and venous blood, particularly the differences in hydrogen ion concentration, may play an important part in controlling the stability of the plasmatic colloidal equilibrium and in accounting for the fact that atheromatosis is in general restricted to the arterial part of the vascular tree. The relations existing in this respect may be analogous to those influencing the precipitation of calcium from the plasma, as here also the alkaline state of the arterial blood favors such a process in the arterial wall and in the left side of the heart while the more acid state of the venous blood tends to keep calcium salts in solution. Inasmuch as necrotic tissue usually has an alkaline reaction and in view of the fact that cholesterol membranes in the interface of the plasma and the intima or within the intima favor the development of degenerative changes in the vascular wall, it appears to be possible that disturbances of the cholesterol metabolism and of the plasmatic colloidal lipid equilibrium play an important causal role in the production of the mediocalcinosis of the peripheral arteries, particularly those of the lower extremities.

The importance of a primary colloidal status of the atheromatogenic substances in the plasma for the production of the vascular atheromatous lesions is illustrated additionally by the fact that these agents when present in the plasma in non-colloidal dispersion, i. e., as a coarse emulsion, do not elicit foam cell intimal reactions but foreign body giant cell granulomas which especially affect the small vessels and capillaries of the lung but occasionally also other parts of the vascular tree, such as the testicular venous plexus (Hirsch and Weinhouse, Hirsch, Seemann, Merkulow, Hueper). The lung apparently acts here as a filtration organ in which the globules of lipoids or macromolecular carbohydrates are arrested and reacted on.

#### COLLOID PLASMATIC DISTURBANCES B LIPOIDAL TYPE

- Adam, H. *Ztschr f klin Med* **68** 177, 1909  
 Addis, T., and Oliver, J. *The Renal Lesions in Bright's Disease*, New York, Paul B. Hoeber, Inc., 1931  
 Alpers, B. J. *Arch Neurol & Psychiat* **42** 1173, 1939  
 Alwall, N. *Acta med Scandinav* **101** 83, 1939  
 Anderson, H. *Arch Int Med* **37** 297, 1926

- Anitschkow, N. *Beitr z path Anat u z allg Path* **56** 379, 1913, **57** 201, 1914, **59** 306, 1914, **70** 265, 1922, *Virchows Arch f path Anat* **220** 233, 1915, **249** 73, 1924, *Klin Wchnschr* **4** 2233, 1925, **13** 1259, 1934, *Verhandl d deutsch path Gesellsch* **20** 149, 1925, **23** 473, 1928  
 —and Chalatow, S. *Zentralbl f allg Path u path Anat* **24** 1, 1913  
 Apollonio, C. *Beitr z path Anat u z allg Path* **3** 290, 1888  
 Arning, E., and Lippmann, A. *Ztschr f klin Med* **89** 107, 1920  
 Aschoff, L. *Virchows Arch f path Anat* **235** 152, 1921, *Beihfte z Med Klin* (no 1) **26** 1, 1930  
 Astwood, E. B. *J Pharmacol & Exper Therap* **78** 79, 1943  
 —Sullivan, J., Bissell, A., and Tyslowitz, R. *Endocrinology* **32** 210, 1943  
 Aylward, F. X., and Stott, W. *Biochem J* **31** 2055, 1937  
 Bailey, C. H. *Proc Soc Exper Biol & Med* **13** 60, 1915  
 Baker, L. E., and Carrell, A. *J Exper Med* **45** 305, 1927  
 von Balo, J. *Beitr z path Anat u z allg Path* **102** 341, 1939  
 Balzer, F. *Arch Physiol* **3-4** 65, 1884  
 Bang, I. *Biochem Ztschr* **91** 86, 104, 111 and 122, 1920  
 Barkan, G. *Munchen med Wchnschr* **79** 621, 1932  
 Barreda, P. *Klin Wchnschr* **13** 290, 1934  
 Baumann, C. A., and Rusch, H. P. *Proc Soc Exper Biol & Med* **38** 647, 1938  
 Baumann, T. *Klin Wchnschr* **14** 1743, 1935  
 Bayer, G., and Gaisbock, F. *Wien med Wchnschr* **74** 2001, 1924  
 Beitzke, H. *Virchows Arch f path Anat* **267** 625, 1928, **275** 532, 1930  
 Bell, E. T. *Am J Path* **10** 705, 1934  
 Benda, C. *Virchows Arch f path Anat* **254** 600, 1925  
 Beneke, R. *Frankfurt Ztschr f Path* **28** 407, 1922, *Beitr z path Anat u z allg Path* **87** 285, 1931, *Verhandl d deutsch path Gesellsch* **35** 251, 1935  
 Beutel, A. *Strahlentherapie* **69** 400, 1941  
 Bianchi, E. *Med d lavoro* **29** 213, 1938  
 Black, H. A., and Kampmeier, R. H. *Colorado Med* **31** 262, 1934  
 Blackman, S. S. *Bull Johns Hopkins Hosp* **55** 1, 1934  
 Bloom, D., Kaufman, S. R., and Stevens, R. A. *Arch Dermat & Syph* **45** 1, 1942  
 Bloor, W. R. *J A M A* **119** 1018, 1942  
 Blotner, H. *New England J Med* **203** 709, 1930  
 Blum, F. *Schweiz med Wchnschr* **71** 1612, 1941  
 Blumer, C., Gordonoff, T., and Reznikoff, L. *Arch f exper Path u Pharmakol* **173** 42, 1933  
 Boehm, R., and Berg, F. *Arch f exper Path u Pharmakol* **5** 329, 1876  
 Boggs, T. R., and Morris, R. S. *J Exper Med* **11** 553, 1909  
 Bollman, J. L., and Flock, E. V. *Arch Path* **28** 762, 1939, *Proc Am Soc Exper Path*, 1941  
 Bork, K. *Virchows Arch f path Anat* **262** 646, 1926  
 Boruttau. *Ztschr f exper Path u Therap* **18** 203, 1916  
 Bourneville. *Arch d Neurol* **16** 37, 97, 241 and 346, 1903  
 Broking. *Ztschr f exper Path u Therap* **8** 125, 1911  
 Bronstein, I. P. *J A M A* **100** 1661, 1933  
 Bross, K. *Virchows Arch f path Anat* (supp.) **227** 145, 1920

- Broun, G O, and Muether, B O J A M A **118** 1403, 1942
- Bruger, M J Biol Chem **108** 463, 1935
- and Fitz, F Arch Path **25** 637, 1938
- and Rosenkrantz, J A J Clin Endocrinol **2** 176, 1942
- Wright, I S, and Wiland, J Arch Path **36** 612, 1943
- Brunner, W Klin Wchnschr **14** 1853, 1935
- Buchholtz Arch f exper Path u Pharmacol **82** 30, 1918
- Burger, M Verhandl d Gesellsch f Verdauungskr **14** 88, 1938, Klin Wchnschr **17** 1780, 1938, Verhandl d deutsch path Gesellsch **31** 88, 1938
- and Habs, H Klin Wchnschr **6** 2221, 1927
- and Schlomka, G ibid **7** 1944, 1928
- Burgi, E Munchen med Wchnschr **74** 2019, 1927
- Buschke, A, Klopstock, E, and Peiser, B Med Klin **20** 345, 1924
- Calvin, J C, and Goldberg, A H Am J Dis Child **41** 1066, 1931
- Chanka, T V Arkh biol nauk **57** 74, 1940
- Chaikoff, I L, Entenman, C Changus, G W, and Reichert, F L Endocrinology **28** 797, 1941
- Chalatow, S S Zentralbl f allg Path u path Anat **25** 197, 1914, Virchows Arch f path Anat **207** 452, 1912, **272** 691, 1929
- Chanutin, A, and Ludewig, S J Biol Chem **102** 57, 1933
- Chiari, H Verhandl d deutsch path Gesellsch **9** 340, 1905
- Cholesterolesmia and Thyroid Disorder, editorial, J A M A **103** 113, 1934, J Clin Endocrinol **3** 526, 1943
- Chuma, M Virchows Arch f path Anat **242** 275, 1923
- Cirio, L Virchows Arch f path Anat **269** 739, 1928
- Clark, H C Am J Dis Child **59** 353, 1940
- Clarkson, S, and Newburgh, L H J Exper Med **43** 595, 1926
- Collens, W S, and Wilensky, N D J A M A **109** 2125, 1937
- Cook, R P Biochem J **30** 1630, 1936, Nutrition Abstr & Rev **12** 1, 1942
- and McCullagh, G P Quart J Exper Physiol **29** 283, 1939
- Corcoran, A C, and Rabinowitch, I M Biochem J **31** 343, 1937
- Corwin, W C Arch Path **26** 456, 1938
- Craig, L S, Lissner, H, and Soley, M H J Clin Endocrinol **4** 12, 1944
- Damrau, F M Rec **144** 373, 1936
- Dansch, F Klin Wchnschr **7** 289 and 337, 1928, Beitr z path Anat u z allg Path **79** 333, 1928
- Dauber, D V, and Katz, L N Arch Path **34** 937, 1942, **36** 473, 1943
- Dauksys, J J Missouri M A **32** 466, 1935
- Davis, D, Stern, B, and Lesnick, G Ann Int Med **11** 354, 1937
- Davis, H H, and Warren, S Arch Path **16** 853, 1933
- De Courcy, J L J Med **9** 9, 1928
- Degwitz, R Klin Wchnschr **9** 2336, 1930
- Deicke, O Krankheitsforsch **3** 399, 1926
- De Langen, C D Acta med Scandinav **97** 427, 1938
- Determann Deutsche med Wchnschr **34** 871, 1908
- and Broking ibid **38** 994, 1912
- Diez, S Beitr z path Anat u z allg Path **83** 74, 1929
- Dirr K Klin Wchnschr **18** 91, 1939
- Domagk, G Klin Wchnschr **17** 1780, 1938
- and von Dohnneck, P Virchows Arch f path Anat **290** 385, 1933
- Dominguez, R J Exper Med **46** 463, 1927
- Domisch Klin Wchnschr **6** 1781, 1926
- Dormanns, E, and Emminger, E Virchows Arch f path Anat **295** 525, 1935
- Dragstedt, L R J A M A **114** 29, 1940, Am J Physiol **129** P348, 1940
- Clark, D E, Julian, O C, Vermeulen, C, and Goodpasture, W C Surgery **8** 353, 1940
- Goodpasture, W C, Vermeulen, C, and Clark, D E Am J Physiol **126** P479, 1939
- Drv, T J, and Hines, E A Ann Int Med **14** 1893, 1941
- Dublin, L I, and Iotka, A J Twenty-Five Years of Health Progress, New York, Metropolitan Life Insurance Co, 1937, p 319
- Dull, L Arch Path **20** 81 and 259, 1935, **21** 161, 1936
- Duncan, A G J Ment Sc **77** 332, 1931
- Dungl, N P Lancet **1** 1354, 1936
- Dupce, C, Johnson, V, Marchello, A, Wilner, W, and Freeman, L W Federation Proc **3** 8, 1944
- Eberhard, T P Arch Path **21** 616, 1936
- Ehrenthel, O, and Weis-Ostborn, W Ztschr f Immunitätsforsch u exper Therap **36** 356, 1923
- von Eiselsberg Deutsche Ztschr f Chir **73** 47, 1904
- Arch f klin Chir **49** 207, 1895
- Engelberg, H, and Newman, B A J A M A **122** 1167, 1943
- English, J P, and Willius, F A Arch Int Med **71** 594, 1943
- Eppinger, H Verhandl d deutsch path Gesellsch **31** 51, 1938
- Epstein, A A J Exper Med **20** 334, 1914, J A M A **69** 444, 1917, Am J M Sc **154** 638, 1917, **163** 167, 1922, Arch f Verdauungskr **44** 31, 1928
- Epstein, E Virchows Arch f path Anat **281** 152, 1931
- Epstein, E Z, and Greenspan, E B Arch Int Med **58** 860, 1936
- Ernst, P Beitr z path Anat u z allg Path **63** 141, 1916
- Fagge, C H Tr Path Soc London **24** 242, 1873
- Fahr, T Verhandl d deutsch path Gesellsch **15** 234, 1912, Klin Wchnschr **13** 609, 1934
- Falta, W Die Erkrankungen der Schilddrüse, in Mohr, L, and Staehelin, R Handbuch der inneren Medizin, ed 2, Berlin, Julius Springer, 1927 vol 4, pt 2, p 1035
- Fenz, E, and Zell, F Klin Wchnschr **36** 1133, 1936
- Ferraro, A, Jervis, G A, and Flicker, D J Arch Path **32** 723, 1941
- Filatoff and Rachmaninoff Jahrb f Kinderh **19-20** 209, 1883
- Fischer, W Deutsches Arch f klin Med **97** 230, 1909
- Fishberg, A M J A M A **82** 463, 1924
- Fleischmann, W, and Shumacker, H B, Jr Bull Johns Hopkins Hosp **71** 175, 1942
- Shumacker, H B, Jr, and Wilkins, L Am J Physiol **131** 317, 1940
- and Wilkins, L J Clin Endocrinol **1** 799, 1941
- Fleener, J, Bruger, M, and Wright, I S Arch Path **31** 82, 1941
- Flock, E V, Corwin, W C, and Bollman, J L Am J Physiol **123** 558, 1938
- Fox, C Lancet **2** 688, 1879
- Freund, H, and König, W Arch f exper Path u Pharmacol **133** 317, 1928

- Freyberg, R H Arch Int Med **59** 660, 1937
- Friedberg, L, and Hurwitz, M Federation Proc **1** 26, 1942
- Friedland, I B Ztschr f d ges exper Med **87** 683, 1933
- Froboese Zentralbl f allg Path u path Anat **31** 225, 1920-1921
- Futcher, T B Ann de dermat et syph **7** 887, 1906, Am J M Sc **130** 939, 1905
- Gallego, J D Arch f Gewerbepath u Gewerbehyg **8** 124, 1938
- Garb, J Ann Int Med **24** 241, 1943
- Gardner, J A Brit M J **2** 392, 1932
- Gate, J, Chamal, G, Vallet, A, and Humbert, P Ann de dermat et syph **9** 465, 1938
- Geiling, E M K, Jensen, H, and Farrar, G E Insulin, in Heffter, A Handbuch der experimentellen Pharmakologie, Berlin, Julius Springer, 1935, vol 5, p 279
- Ghaloungui, P, and Zell, F Arch f exper Path u Pharmakol **185** 71, 1937
- Gibbs, C B F, Buckner, E, and Bloor, W R New England J Med **209** 384, 1933
- Gilbert, G G Arch Int Med **68** 591, 1941
- Gildea, E F, Man, E B, and Peters, J P J Clin Investigation **18** 739, 1939
- Gorog, D Virchows Arch f path Anat **287** 602, 1933
- Goldmann, E Beitr z klin Chir **18** 595, 1897
- Gordy, S T, and Trumper, M J A M A **110** 1543, 1938, Indust. Med **9** 231, 1940
- Goreczky, L, and Kovats, J Biochem Ztschr **314** 208, 1943
- Greene, A M Arch Int Med **67** 114, 1941
- Grigaut, A, and L'Huillier, A Compt rend Soc de biol **73** 304, 1912
- Gross, P, and Jacob, H W Am J M Sc **203** 673, 1942
- Grotel, D M, Bykhovskaya, E E, Pavlova, M M, Pokhodilova, M G, and Shor, V G Klin Med **18** 34, 1940
- Gruenfeld, G, and Seelig, M G Arch Path **17** 546, 1934
- Grutz, O Klin Wchnschr **17** 1780, 1938, Verhandl d deutsch path Gesellsch **31** 81, 1938, Verhandl d Gesellsch f Verdauungskr **14** 81, 1938
- Guggenheimer, H, and Fisher, I L Ztschr f d ges exper Med **54** 114, 1927
- Guttmann, E Klin Wchnschr **6** 1808, 1927
- Gwynne, C N Rep Soc Study Dis Child **5** 318, 1905
- Gyorgy, P Klin Wchnschr **3** 483, 1924, Zentralbl f Kinderh **112** 283, 1926
- Hamperl, H Virchows Arch f path Anat **271** 147, 1929
- Handovsky, H Klin Wchnschr **3** 1354, 1924, **10** 1158, 1931, Arch f exper Path u Pharmakol **134** 191, 1928, **137** 264, 1928, **159** 383, 1931
- and von Trossel, I Skandinav Arch f Physiol **49** 145, 1926
- Harbitz, F Norsk mag f lægevidensk **86** 321, 1925, **97** 695, 1936, **98** 1317, 1937, **99** 1250, 1938, Arch Path **4** 507, 1927
- Harrison, C V J Path & Bact **36** 447, 1933
- Havthorn, S R, Taylor, F A, Crago, H W, and Burrier, A Z Am J Path **12** 283, 1936
- Heckscher, H Biochem Ztschr **158** 417 and 422, 1925
- Heme, J Verhandl d deutsch path Gesellsch **27** 78, 1934, Beitr z path Anat u z allg Path **94** 412, 1935
- Hellwig, C A Am Heart J **24** 41, 1942
- Herman, K Orvosi hetil **78** 1208, 1934
- Herrmann, E, and Neumann, J Wien klin Wchnschr **25** 1557, 1912
- Hertzka, E Berl klin Wchnschr **18** 567, 1881
- Herzog, E Beitr z path Anat u z allg Path **85** 707, 1931
- Hess, F O Verhandl d deutsch Gesellsch f Kreislaufforsch **7** 57, 1934, Klin Wchnschr **13** 714, 1934
- Hesse, E Klin Wchnschr **18** 502, 1939
- Hesse, M Virchows Arch f path Anat **261** 225, 1926
- Heyn, F Arch f Psychiat **41** 49, 1905
- Himsworth, H P Acta med Scandinav (supp) **90** 158, 1938
- Hines, E A, Jr, and Barker, N W Am J M Sc **200** 717, 1940
- Hirsch, E F Arch Path **20** 665, 1935, **21** 764, 1936, **25** 35, 1938
- and Weinhouse, S ibid **30** 1079, 1940, Physiol Rev **23** 185, 1943
- Hoelzer, H Beitr z path Anat u z allg Path **104** 289, 1940
- Hofmeyer, J Nord med **9** 365, 1941
- Hofmeister, E Beitr z klin Chir **11** 441, 1894
- Hoin H, and Finkelstein, L E Am Heart J **19** 655, 1940
- Huber, M J, Broun, G O, and Casey, A E Proc Soc Exper Biol & Med **37** 441, 1937
- Hueck W Verhandl d deutsch path Gesellsch **20** 18 1925, Zentralbl f allg Path u path Anat **23** 454, 1912
- Hueper, W C Arch Path **38** 93 and 350, 1944, **40** 51, 1945
- Hughes, F W T, and Perry, C B Bristol Med-Chir J **46** 219, 1929
- Hunt, H M New England J Med **201** 659, 1929
- Hurwitz, M, and Friedberg, L Arch Path **34** 875, 1942
- Hurxthal, L M Arch Int Med **53** 762 and 825, 1934
- and Hunt, H M Ann Int Med **9** 717, 1935
- and Simpson, H N J Clin Endocrinol **1** 450, 1941
- Ignatowski, A I Izviest Imp Voenno-Med Akad **16** 154, 1908
- Isenschmid Pathologische Physiologie der Schilddrüse, in Bethe, A, von Bergmann, G, Embden, G, and Ellinger, A Handbuch der normalen und pathologischen Physiologie, Berlin, Julius Springer, 1930, vol 16, pt 1
- Jobling, J W, and Meeker, D R Arch Path **21** 293, 1936
- Joel, E Klin Wchnschr **3** 269, 1924
- Johnson, V, Freeman, L W, Longini, J, and Loewy, A Federation Proc **3** 22, 1944
- Joslin, E P Ann Int Med **4** 54, 1930, Trauma and Diabetes Mellitus, in Brahdry, L, and Kahn, S Trauma and Disease, ed 2, Philadelphia, Lea & Febiger, 1941, p 536, The Treatment of Diabetes Mellitus, ed 6, ibid, 1937
- Katsch, G, and Kramick, H G Klin Wchnschr **18** 436, 1939
- Katz, L N, Sanders, A, Megibow, R S, and Carlen, S Am J M Sc **200** 731, 1940
- Kauvar, A J J Clin Endocrinol **1** 955, 1941
- Kennedy, T H Nature, London **150** 233, 1942
- Kesten, H D, and Silbowitz, R Proc Soc Exper Biol & Med **49** 71, 1942

- Keys, A, and Butt, H R Arch Int Med **63** 165, 1939
- Kimmelstiel, P Virchows Arch f path Anat **282** 402, 1931
- Kirch, E Beitr z path Anat u z allg Path **70** 75, 1922
- Kirchgessner, G Klin Wchnsch **13** 976, 1934
- Klotz, O Am J Path **10** 700, 1934
- Knack, A V Virchows Arch f path Anat **220** 36, 1915
- Knauer, H Klin Wchnsch **8** 1745, 1929, Ergebnisse der Lipidstoffwechselforschung mit besonderer Berücksichtigung der Verhältnisse im Kindesalter, Berlin, S Karger, 1928
- Kofler, L, and Fischer, R Arch f exper Path u Pharmacol **141** 105, 1929
- Kolen, A A Virchows Arch f path Anat **263** 46, 1927, **272** 679, 1929
- Kollert, V, and Rezek, P Virchows Arch f path Anat **262** 838, 1926
- and Grill, H Ztschr f d ges exper Med **49** 522, 1926
- Kofler, L, and Susani, O ibid **45** 682, 1925
- Koppenhofer, G F Virchows Arch f path Anat **297** 271, 1936
- Kraus, H Arch f exper Path u Pharmacol **179** 537, 1935
- Krisch, H Virchows Arch f path Anat **230** 191, 1921
- Krylow, D Compt rend Soc de biol **79** 397 and 399, 1916
- Kube, N, and Ssolowjew, A Frankfurt Ztschr f Path **40** 302, 1930
- Kuczynski, B Klin Wchnsch **4** 39, 1925
- Kusnetzowski, N J Zentralbl f allg Path u path Anat **32** 534, 1921-1922
- Lande, K E, and Sperry, W M Arch Path **22** 301, 1936
- Lane, G C, and Goodman, J, Jr Arch Dermat & Syph **32** 377, 1935
- Lapowski, B Arch Dermat & Syph **11** 701, 1925
- Lasch, F Ztschr f d ges exper Med **42** 548, 1924, Klin Wchnsch **13** 1534, 1934
- Lauritzen, K Virchows Arch f path Anat **279** 603, 1930
- Leary, T New England J Med **205** 231, 1931, Arch Path **17** 453, 1934, **21** 459, 1936, **29** 665, 1940, **32** 507, 1941, **37** 16, 1944, Am Heart J **16** 549, 1938, West J Surg **48** 537, 1940, J A M A **124** 385, 1944
- von Leersum, M Virchows Arch f path Anat **217** 452, 1914
- Lehndorff, A Arch f exper Path u Pharmacol **76** 224, 1914
- Lehnher, E R New England J Med **208** 1307, 1933
- Lehzen, G, and Knauss, K Virchows Arch f path Anat **116** 84, 1889
- Lerman, J J A M A **117** 349, 1941
- Letterer, E Verhandl d Gesellsch f Verdauungsk **14** 12, 1938, Verhandl d deutsch path Gesellsch **31** 12, 1938
- Levy, H, and Boas, E P J A M A **107** 97, 1936
- Lewey, F H Ann Int Med **15** 869, 1941, J Indust Hyg **23** 415, 1941
- Li, T-W, Hough, V H, Monahan, E P, and Freeman, S Federation Proc **2** 50, 1943
- Lichtenstein, L, and Epstein, E Z Arch Int Med **47** 122, 1931
- Liebe, S Arch f exper Path u Pharmacol **171** 426, 1933
- Liebig, H Klin Wchnsch **8** 1516, 1929, **10** 475, 1931, **20** 538, 1941, Arch f exper Path u Pharmacol **159** 265 and 359, 1931, **175** 409, 1934
- Lisa, J R, Magiday, M, Galloway, I, and Hart, J F J A M A **120** 192, 1942
- Magiday, M, and Hart, J F ibid **118** 1353, 1942
- Llosa Ricketts, G Rev peruana de pediat **1** 1, 1942
- Loeb, O Arch f exper Path u Pharmacol **69** 108, 1912
- and Michaud Biochem Ztschr **3** 367, 1907
- Lowenthal, K Beitr z path Anat u z allg Path **61** 564, 1915-1916, **79** 497, 1928, Verhandl d deutsch path Gesellsch **20** 137, 1925, **21** 209, 1926, Frankfurt Ztschr f Path **34** 145, 1926, Klin Wchnsch **5** 478, 1926, Virchows Arch f path Anat **251** 108, 1926
- Longini, J, and Johnson, V Am J Physiol **140** 349, 1943
- Ludden, J B, Bruger, M, and Wright, I S Endocrinology **28** 999, 1941, Arch Path **33** 58, 1942
- McArthur, C S Biochem J **36** 559, 1942
- McDonald, R Arch Ophth **20** 839, 1938
- Mackenzie, C G, and Mackenzie, I B Endocrinology **32** 185, 1943
- Mackenzie, J B, and Mackenzie, C G Federation Proc **1** 122, 1942
- Mackenzie, C G, and McCollum, L V Science **94** 518, 1941
- Malisoff, W M Proc Soc Exper Biol & Med **35** 356, 1936
- Man, E B, and Peters, J P J Clin Investigation **12** 1031, 1933
- Mancke, R Arch f exper Path u Pharmacol **149** 56, 1930
- Marchand, F Verhandl d Kong f inn Med **21** 23, 1904
- Maresch R Ztschr f Heilk **19** 249, 1898
- Marine, D Arch Path **28** 65, 1939
- Martius, K, Frankfurt Ztschr f Path **5** 515, 1910, **15** 135, 1914
- Masing, E Fortschr d Therap **7** 193, 1931
- Mason, R L, Hunt, H M, and Hurvath, L New England J Med **203** 1273, 1930
- Masson, P Schweiz med Wchnsch **71** 1042 1941
- Matusita, T Jap J M Sc, IV Pharmacol **12** 38, 1940
- Medvei, C V Klin Wchnsch **11** 414, 1932
- Meeker, D R, and Jobling, I W Arch Path **18** 252, 1934
- and Kesten, H D ibid **31** 147, 1941, Proc Soc Exper Biol & Med **45** 543, 1940
- Kesten, H D, and Jobling, J W Arch Path **20** 337, 1935
- Member, S, Ehrlich, S B, and Bruger, M Proc Soc Exper Biol & Med **46** 560, 1941
- Menne, F R, Beeman, J A, and Labby, D H Arch Path **24** 612, 1937
- Merkel, H Beitr z path Anat u z allg Path **104** 332, 1940
- Merkulow, G A Virchows Arch f path Anat **286** 571, 1932
- Messina, R Folia med **17** 1225, 1931
- Moehlig, R C Endocrinology **14** 337, 1930
- and Osius, E A Ann Int Med **4** 579, 1930
- Monckeberg, J G Virchows Arch f path Anat **167** 191, 1902
- Moll, T, Domagk, G, and Laquer, F Klin Wchnsch **12** 465, 1933
- Montgomery, H Proc Staff Meet, Mayo Clin **12** 641, 1937

- Moon, V H Arch Path **3** 404, 1927
- Morawitz, P, and Pratt, J Munchen med Wchnschr **55** 1817, 1908
- Moschcowitz, E Vascular Sclerosis, New York, Oxford University Press, 1942
- Mosebach, W Virchows Arch f path Anat **289** 646, 1933
- Moxon Tr Path Soc London **24** 129, 1873
- Muller, C Acta med Scandinav **89** 75, 1938, Nord med **2** 1183, 1939, Arch Int Med **64** 675, 1939
- Muller, O, and Inada, R Deutsche med Wchnschr **30** 1751, 1904
- Muller, G L Medicine **9** 119, 1930
- Murata, M, and Kataoka, S Verhandl d jap path Gesellsch **7** 27, 1917, **8** 221, 1918
- Murchison Tr Path Soc London **20** 187, 1869
- Natali, C Frankfurt Ztschr f Path **47** 1, 1934
- Nekam, L, and Ottenstein, B Klin Wchnschr **14** 641, 1935
- Nelson, M G J Path & Bact **53** 105, 1941
- Newburgh, L H, and Clarkson, S J A M A **79** 1106, 1922, Arch Int Med **31** 653, 1923
- Nikuln, M, and Hetmann, Z Arch f Gewerbepath u Gewerbehyg **4** 653, 1933
- Nisi, H Tr Soc path jap **29** 272, 1939
- Nuzum, F R, Seegal, B, Garland, R, and Osborne, M Arch Int Med **37** 733, 1926
- Nyboer, J, Bruger, M, and Rabson, S M Am Heart J **21** 657, 1941
- Okey, R, and Greaves, V D J Biol Chem **129** 111, 1939
- and Stewart, D ibid **99** 717, 1933
- Okuneff, N Beitr z path Anat u z allg Path **71** 99, 1922-1923
- Onizawa, J J Biochem **10** 425, 1939
- Ophuls, W The Pathogenesis of Arteriosclerosis, in Cowdry, E V Arteriosclerosis, New York, The Macmillan Company, 1933
- Pachorukow, D Arb d pharmakol Inst zu Dorpat **1** 1, 1888
- Page, I H Ann Int Med **14** 1741, 1941
- and Bernhard, W G Arch Path **19** 530, 1935
- and Farr, L E J Clin Investigation **15** 181, 1936
- and Menschick, W Naturwissenschaften **18** 585, 1930
- Kirby, E, and Van Slyke, D D J Clin Investigation **15** 101, 1936
- Pagel, W Virchows Arch f path Anat **258** 416, 1925
- Paterson, J C Arch Path **22** 313, 1936
- Pekelharing, C A Beitr z path Anat u z allg Path **8** 245, 1890
- Peters, J P, and Man, E B J Clin Investigation **22** 715 and 721, 1943
- Petersilie, H J Lab & Clin Med **20** 144, 1934
- Pick, E P, and Pineles, F Ztschr f exper Path u Therap **7** 518, 1909
- Pick, L Deutsche med Wchnschr **37** 1930, 1978, 2036 and 2086, 1911
- Pilgersdorfer, W Klin Wchnschr **19** 512, 1940
- Pineles, F Mitt a d Grenzgeb d Med u Chir **14** 120, 1904-1905
- Poensgen, A Virchows Arch f path Anat **91** 350, 1883
- Polano, M K Arch f Dermat u Syph **174** 213, 1936
- Popken, C Beitr z path Anat u z allg Path **97** 337, 1936
- Pye-Smith, P H Tr Path Soc London **24** 250, 1873, Guy's Hosp Rep **22** 97, 187,
- Raab, W Munchen med Wchnschr **86** 689, 1939
- and Friedmann, R Klin Wchnschr **16** 1159, 1936
- Rabinowitch, I M Ann Int Med **8** 1436, 1935
- Richie, W I, and McKee, S H ibid **7** 1478, 1934
- Rabl, R Virchows Arch f path Anat **266** 133, 1927-1928
- Radwin, L S Am J Dis Child **60** 1120, 1940
- Ranke Beitr z path Anat u z allg Path **71** 78, 1923, **75** 269, 1926
- Redfield, R L J Am Inst Homeop **33** 584, 1940
- Reineck, H Beitr z path Anat u z allg Path **80** 145, 1928
- Remesow Verhandl d deutsch path Gesellsch **37** 142, 1934, Zentralbl f allg Path u path Anat **49** 361, 1930
- and Tavaststerna, N Ztschr f d ges exper Med **76** 419, 1931
- Remond, A, Colombies, H, and Bernardberg, J Compt rend Soc de biol **91** 445, 1924
- Richter, C P, and Clisby, K H Proc Soc Exper Biol & Med **48** 684, 1941
- Roemer, R Ztschr f Geburtsh **71** 350, 1912
- Rossle Kon-Bl d allg arztl Ver v Thuringen **49** 21, 1920
- Rohrschneider, W Virchows Arch f path Anat **256** 139, 1925
- Romberg, E Verhandl d Kong inn Med **21** 90, 1904, Lehrbuch der Krankheiten des Herzens und der Blutgefasse, Stuttgart, F Enke, 1921
- Root, H F, Bland, E F, Gordon, W H, and White, P D J A M A **113** 27, 1939
- Rosenthal, F, and Meier, K Arch f d ges exper Path **91** 246, 1921
- Friedlander, E, and Kohn, R Arch f exper Path u Pharmakol **175** 343, 1934
- Rosenthal, S R Arch Path **18** 473, 660 and 827, 1934
- Sakai, S Biochem Ztschr **62** 387, 1914
- Sakurai, S Jap J Exper Med **7** 449, 1929
- Saltykow, S Verhandl d deutsch path Gesellsch **14** 119, 1910, **21** 398, 1926, Virchows Arch f path Anat **213** 8, 1913, Beitr z path Anat u z allg Path **57** 415, 1913
- Sanders Am J M Sc **142** 727, 1911
- Sappington, S W, and Fisher, H R Arch Path **34** 989, 1942
- and Horneff, J A Am J M Sc **201** 862, 1941
- Saxton, J A New York State J Med **41** 1095, 1941
- Scarff, R W J Path & Bact **30** 647, 1927
- Schally, A O Ztschr f klin Med **128** 376, 1935
- Scheel, O Virchows Arch f path Anat **191** 135, 1908
- Schlossman, N C, and Gerber, L Ann Surg **115** 292, 1942
- Schmetz Klin Wchnschr **15** 805, 1936
- Schmidt, L H, and Hughes, H B Endocrinology **22** 474, 1938
- Schmidtmann, M Virchows Arch f path Anat **237** 1, 1922, **255** 206, 1925, Verhandl d deutsch path Gesellsch **22** 226, 1927
- and Huttich, M Virchows Arch f path Anat **267** 601, 1928
- Schonheimer, R Ztschr f physiol Chem **160** 61, 1926, **177** 143, 1928, **211** 65, 1932, Klin Wchnschr **43** 1793, 1932, Virchows Arch f path Anat **249** 1, 1924, **251** 732, 1924
- and Yuasa, D Verhandl d deutsch path Gesellsch **25** 304, 1927
- Schottmuller, H Klin Wchnschr **10** 620, 1931



- Schulte, E Beitr z path Anat u z allg Path **61** 570, 1915-1916
- Schultz, A Virchows Arch f path Anat **239** 415, 1922, Verhandl d deutsch path Gesellsch **20** 120, 1925
- Scupham, G W, de Takats, G, Van Dellen, T R, and Beck, W C Arch Int Med **64** 590, 1939
- Seel, H, and Creuzberg, G Arch f exper Path u Pharmakol **161** 674, 1931
- Seemann, G Beitr z path Anat u z allg Path **83** 705, 1930
- Shapiro, S M J & Rec **126** 284, 1927, J Exper Med **45** 595, 1927, Endocrinology **11** 279, 1927
- and Seecoff, D P J Lab & Clin Med **10** 826, 1925
- Shelton, E K J A M A **117** 1948 1941
- Shulito, F H, Bidwell, E H, and Turner, K B J Biol Chem **112** 551, 1936
- Siegmund Verhandl d deutsch path Gesellsch **18** 59, 1921
- Simnitzky, S Ztschr f Heilk **24** 177, 1903
- Smetana, H Virchows Arch f path Anat **274** 170, 1929, **274** 697, 1930
- Smith, W F Tr Path Soc London **28** 236, 1877
- Sobotka, H Macromolecular Layers Their Application in Physiology and Medicine, in Glasser, O Medical Physics, Chicago, The Year Book Publishers, Inc, 1944, p 763
- Sperry, W M, in Luck, J M Annual Review of Biochemistry, Stanford University, Calif, Annual Reviews, Inc, 1939, vol 8 p 231
- and Stoyanoff, V A J Nutrition **9** 131 and 157, 1935
- Sokoloff, N A Virchows Arch f path Anat **245** 203, 1923
- Ssolojew, A Virchows Arch f path Anat **241** 1 1923, **250** 360, 1924, **261** 253, 1926, **283** 213, 1932, Zentralbl f allg Path u path Anat **53** 145, 1932
- Stembiss, W Virchows Arch f path Anat **212** 152, 1913
- Steiner, A Proc Soc Exper Biol & Med **38** 231 1938 **39** 411, 1938
- and Domanski, B Am J M Sc **201** 820, 1941 **204** 79, 1942, Arch Int Med **71** 397, 1943, Proc Soc Exper Biol & Med **55** 236, 1944
- Stepp, W Beitr z path Anat u z allg Path **69** 233, 1921
- Stern, R Arch f exper Path u Pharmakol **112** 129, 1926
- Stewart, H J, and Evans, W F Am Heart J **23** 175, 1942
- Stockman and Charteris Brit M J **2** 1520, 1901
- Stokes, E H A Clinical and Experimental Investigation of the Blood Cholesterol Content in Myxoedema and Other Conditions Sydney, Australasian Medical Publishing Co, Ltd, 1941
- Strauss, H Acta med Scandinav **93** 526, 1937
- Strauss, L H Ztschr f exper Med **98** 603, 1936
- and Scheer, P Klin Wchnschr **15** 187, 1936
- Strome, F P, and Blaine, B C Pennsylvania M J **43** 431, 1940
- Strong, R A J A M A **107** 422, 1936
- Stukkeř, N V Alterations in the Aorta of Rabbits Under the Influence of Excessive Animal Diet Dietetic Arteriosclerosis, Inaug Dissert, St Petersburg, 1910, Zentralbl f allg Path u path Anat **21** 668, 1910, **22** 379 1911, **23** 910, 1912
- Stumpf Beitr z path Anat u z allg Path **59** 396, 1914
- Sumikawa, T Virchows Arch f path Anat **196** 232, 1909
- Svendsen, M Acta med Scandinav **104** 235, 1940
- Tammenberg, J Blutgefasse, in Lichtwitz, L, Liesegang, R E, and Spiro, K Medizinische Kolloid lehre, Leipzig Theodor Steinkopff, 1935, p 570
- Thannhauser, S J Verhandl d deutsch path Gesellsch **20** 5, 1925, Lipoidoses Diseases of the Cellular Lipid Metabolism, New York, Oxford University Press, 1940
- and Magendantz, H Ann Int Med **11** 1662 1938
- Thiersch H Beitr z path Anat u z allg Path **97** 81, 1936
- Thollde M Beitr z path Anat u z allg Path **77** 61, 1927
- Thomas, W A J A M A **88** 1559, 1927
- Thompson, K W, and Long, C N H Endocrinology **28** 715, 1941
- Thompson, W O J A M A **117** 441, 1941
- Tregubow, A Krankheitsforsch **6** 87, 1928
- Tropp, C Klin Wchnschr **15** 562, 1936
- Turner K B J Exper Med **58** 115, 1933
- and Bidwell, E H ibid **62** 721, 1935
- and Khavat, G B ibid **58** 127, 1933
- and Steiner, A J Clin Investigation **18** 45, 1939
- Present, C H, and Bidwell, E H J Exper Med **67** 111, 1938
- Uchiyama, J Virchows Arch f path Anat **277** 642, 1930
- Ungar H Arch f exper Path u Pharmakol **175** 536, 1934
- Vander Veer, J B M Clin North America **23** 1561 1939
- Vermeulen, C W Allen J G Clark D E Julian O C, and Dragstedt, L R Proc Am Physiol Soc, 1941
- Dragstedt L R Clark, D E Julian, O C and Allen, J G Arch Surg **42** 260, 1942
- Versc, M Beitr z path Anat u z allg Path **63** 780 1917, Verhandl d deutsch path Gesellsch **19** 163 1923 **20** 67, 1925 Virchows Arch f path Anat **250** 252 1924 Zentralbl f allg Path u path Anat **34** 614, 1924
- Virchow, R Phlogose und Thrombose im Gefasssystem, in Gesammelte Abhandlungen zur wissenschaftlichen Medizin, Frankfurt a M, Meidinger Sohn & Co, 1856
- Volhard, F Die doppelseitigen hamatogenen Nieren-erkrankungen, in Mohr L and Stachelin, R Handbuch der inneren Medizin, ed 2, Berlin, Julius Springer, 1931, vol 6, pt 1, p 1
- Wacker, L, and Hueck, W Munchen med Wchnschr **60** 2097, 1913 Arch f exper Path u Pharmakol **71** 373, 1912-1913, **74** 416, 1913
- Wada, K Tr Jap Path Soc **16** 179, 1926
- Wail, S S Virchows Arch f path Anat **245** 219 1923
- Warischew, W K Inaug Dissert Warsaw, 1914
- Warren, S The Pathology of Diabetes Mellitus, ed 2, Philadelphia, Lea & Febiger, 1938, p 246
- Wartman, W B Factors Concerned in Narrowing or Occlusion of Coronary Vessels, in Blood, Heart and Circulation, Washington, D C, American Association for the Advancement of Science, 1940 vol 13, p 122
- Weber, F R Cutaneous Xanthoma and Xanthomatosis of Other Parts of the Body Pituitary Xanthomatosis — Xanthomylomata of Tendon Sheaths, etc and the "Cholesterin-Diathesis," London H K Lewis & Company, 1924

- Wegelin Virchows Arch f path Anat **254** 689, 1925, Schilddrüse, in Henke, F, and Lubarsch, O Handbuch der speziellen Anatomie und Histologie, Berlin, Julius Springer, 1925, vol 8, pt 1
- Weinhouse, S Arch Path **35** 438, 1943
- and Hirsch, E F *ibid* **29** 31, 1940, **30** 856, 1940
- Wepler, W Virchows Arch f path Anat **295** 546, 1935
- Wesselkin, N W Virchows Arch f path Anat **212** 225, 1913
- Westphal, K Klin Wchnschr **5** 1995, 1926, Ztschr f klin Med **101** 545, 558, 584 and 566, 1924-1925
- Westra, J J, and Kunde, M M Am J Physiol **103** 1, 1933
- White, P D Diabetes in Childhood and Adolescence, Philadelphia, Lea & Febiger, 1932, p 178, J A M A **113** 27, 1939
- Whitehead, V I E Brit J Exper Path **24** 192, 1943
- Wilens, S L Am J Path **19** 293, 1943
- Wilkins, L, Fleischmann, W, and Block, W J Clin Endocrinol **1** 3 and 91, 1941, J A M A **116** 2459, 1941
- Winternitz, M C Views as to Causes of Coronary Sclerosis, in Blood, Heart and Circulation, Washington, D C, American Association for the Advancement of Science, 1940, vol 13, p 114, Arch Path **28** 124, 1939
- Thomas, R M, and LeCompte, P M The Etiology of Arteriosclerosis, Springfield, Ill, Charles C Thomas, Publisher, 1938
- Wise, F Arch Dermat & Syph **47** 739, 1943
- Wislicki, L Klin Wchnschr **8** 1568, 1929
- Wolff, K Virchows Arch f path Anat **293** 472, 1934
- Wolkoff, K Beitr z path Anat u z allg Path **85** 386, 1930
- Wright, I Arch Surg **40** 163, 1940
- Yuasa, D Beitr z path Anat u z allg Path **80** 570, 1928
- Zeek, P M Am J Path **12** 115, 1936
- Zinserling, W D Beitr z path Anat u z allg Path **71** 292, 1923, Virchows Arch f path Anat **255** 677, 1925, Zentralbl f allg Path u path Anat **24** 627, 1913, **32** 534, 1921-1922
- Zon, L Arch Path **27** 888, 1939
- Zondek, H Lancet **2** 310, 1941.

## COLLOIDAL PLASMATIC DISTURBANCES

## C PROTEINIC TYPE

Disturbances of the quantitative and qualitative composition of the plasma proteins are present in a great number of different diseases (myelomatosis, hyperfibrinogenemia, amyloidosis immune reactions, chemoallergies, hemoglobinemia, nephrosis, leukemia, hepatic cirrhosis, myxedema, venereal lymphogranuloma, kala-azar) and are artificially produced by the intravenous introduction of homologous or heterologous protein solutions (plasma proteins, gelatin, isinglass, ovalbumin, azoproteins) or by the formation of protein complexes with aromatic substances (sulfonamide compounds, Evans blue). Such disturbances may result from shifts in the ratio of the various plasma protein fractions without causing any increase in the total amount of plas-

ma proteins, or from absolute increases or decreases of one fraction, or from the formation of abnormal endogenous proteins (paraproteins) of smaller or larger size than the ordinary ones, or from combination of a normal plasma protein with an endogenous or exogenous chemical complex, or from the introduction of exogenous proteins. Such changes elicit definite alterations in the colloidal equilibrium of the plasmatic proteins and modify their stability in solution and their reactivity to each other and to the proteins contained in the tissues and the tissue fluids.

Among the three normal plasma proteins (fibrinogen, globulin, albumin) which compose about 6 to 7 per cent of the plasma, albumin has the smallest molecular weight (about 70,000) and the most uniform and symmetric molecular shape exerts the greater part of the total colloidal osmotic pressure of the plasma, is least readily denatured, is most finely dispersed, has the largest electric charge, the lowest viscosity, the greatest solubility and stability in solution and represents over 60 per cent of the total plasma protein. Globulin, which like albumin normally has a round-shaped molecule, has a molecular weight of about 120,000, is more asymmetrically built, more labile in solution, more viscous, exerts less colloidal osmotic pressure and occurs in three varieties, alpha, beta and gamma. Fibrinogen has a fibrillar molecule, represents about 5 per cent of the total plasma protein and has the largest molecular weight. Alpha globulin combines with certain carbohydrate groups to form mucoglobulins (Cohn). The plasma proteins occur in the plasma in a hydrated form, i e, by adsorption or hydration they have taken up water and thus have increased their molecular size considerably (Bennhold). The degree and the type of colloidal dispersion of the different plasma proteins may vary considerably, depending on the associated physicochemical conditions of the plasma, and are not reflected by the albumin-globulin ratio (Melnick).

In the hypoproteinemia characterizing lipoid nephrosis fibrinogen and alpha and beta globulins are increased, while gamma globulin and albumin are decreased. In febrile conditions mainly alpha globulin is increased. Immune reactions are associated with hyperproteinemia caused particularly by increases in gamma globulin. Some antibody globulins (horse, pig and cow antipneumococcus euglobulins) may assume enormous sizes through polymerization of smaller units (molecular weight of about 900,000) and exist close to their isoelectric point (Heidelberger). Antibody globulins belong to the gamma variety, from which they may differ by the way in which the polypeptide chain is refolded or recoiled

(Pauling) Hyperproteinemia of immune or other genesis is associated with increased plasma viscosity and accelerated erythrocytic sedimentation and, in severe cases, with pseudoagglutination of erythrocytes (Vignati and Rauchenberg, Foord and Randall, Jeghers and Selesnick, Kiacke and Hoffman)

*Myelomatosis*—The most striking example of plasmatic proteinic imbalances is found in the quantitative and qualitative abnormalities of the plasma proteins occurring in myelomatosis (Shirer, Duncan and Haden, Jacobson, Cantarow, Kagan) Bing stated that myelomatosis is the most frequent cause of severe hyperproteinemia. Schumacher, Williams and Coltrin observed hyperproteinemia in 23.39 per cent of their cases of myelomatosis, Feller and Fowler, in 9 of 52 cases. According to physicochemical and immunologic studies (Kabat, Moore and Gutman, Gutman, Moore, Gutman, McClellan and Kabat, and Kekwick), three different types of myelomatosis can be distinguished on the basis of the patterns of the plasma protein changes. In one type the gamma globulins are mainly increased, in the second type the beta globulins are elevated, while in the third a normal ratio prevails.

In addition to these quantitative deviations in the total plasma proteins and their individual normal fractions, there occurs in myelomatosis in 65 to 80 per cent of the cases (Geschickter and Copeland, Magnus-Levy, Apitz) an abnormal protein, the Bence Jones protein, which coagulates when heated to 65 C, has a molecular weight of about 35,000 to 40,000 and may combine chemically with pseudoglobulins and other proteins (Mahle, Seed and Welke). The investigations of Bayne-Jones and Wilson and of Robinson have shown that this abnormal endogenous protein is antigenic. It is excreted with the urine and may form casts in the renal tubules by being precipitated out of colloidal mixtures by coacervation. Protein crystals are not infrequently found in the renal tissue (Abrikosoff and Wulff, Gunn and Mahle).

The third type of proteinic plasmatic abnormality occurring in myelomatosis is represented by the occasional appearance of a hypermacromolecular, highly viscous globulin of a molecular weight between 162,000 and 200,000 (von Bonsdorff, Groth and Packalen, Shapiro, Ross and Moore, Perlzweig, Delrue and Geschickter, Bing, Wintrobe and Buell). This protein on chilling settles out from the plasma in the lower layer and when dropped into distilled water precipitates in floccules. When heated to 71 C it coagulates. Its presence in the blood is usually associated with circulatory disturbances. Bell

found a highly viscous globulin plugging the glomerular capillaries in cases of myelomatosis. Perlzweig, Delrue and Geschickter proposed that the hyperproteinemia associated with myelomatosis may possibly be a systemic response to protracted intoxication with a foreign protein, considering Bence Jones protein as a foreign protein. In the light of these findings it is considered significant that myelomatosis is often associated with the occurrence of a proteimosis, amyloidosis (Volland, Apitz, Rosenblum and Kirshbaum, Magnus-Levy, Chester, Rosenblatt, Rosenheim and Wright, Randall).

*Noninflammatory Vascular Reactions* (a) *Hyalinosis* Three types of interstitial or conjunctival proteinic deposits are encountered in vascular walls, namely, amyloid, hyaline and fibrinoid. There are definite relations between these three substances according to their behavior with certain metachromatic stains (Letterer, Dietrich). Loeschke considered interstitial hyaline substance as a product of precipitated protein which has a globulin-like character (Muller) becomes adsorbed to collagen fibrils and shares with amyloid and fibrinoid the quality that it is the result of a deposition of concentrated protein gels (Dietrich) which infiltrated as sols from the blood (Anitschkow). Loeschke proposed that in arteriosclerosis a precipitin protein is deposited on the endothelium which is formed by the interaction of an antigenic protein with an antibody globulin, combining to an insoluble protein and precipitated as a hyaline substance. Amyloid represents, according to Loeschke, only a special variety of hyaline substance. If the antibody formation predominates under such circumstances the site of protein precipitation is near the location where the antibodies are produced. When, on the other hand, the antigen predominates, the site of protein precipitation in the tissues where the antigen is elaborated or released is of minor magnitude, as the antigen enters the blood and precipitation then occurs everywhere in the vascular system. If there is an overwhelming predominance of the amount of antigen over the quantity of antibodies available, the precipitation of the protein complex takes place mainly at the site of the antibody production in the spleen and the reticuloendothelial system. Hyalin is formed at places where arteriosclerotic nutritive disturbances cause a degradation of tissues. As lipoids are often in some way bound to protein, they are precipitated with the hyalin and thus become embedded into the hyaline and amyloid matter. While a part of the hyaline deposits in arteriosclerotic vascular walls may be attributed to a summation of the deposi-

tion of hyaline matter occurring during the course of life as a reaction to various diseases passed through, another part of the hyaline substance may be generated in response to specific factors, probably representing proteimic constituents of the blood

The resemblance of hyaline and amyloid vascular deposits is shown by their reactivity to certain stains and by their local distribution in the vascular walls. Both substances occur in non-inflammatory, chronic degenerative types of vascular lesions. These substances, as well as the processes leading to their deposition, therefore lack irritative and toxic properties. The vascular lesions elicited by them are similar to those caused by other nontoxic proteins, such as gelatin and ovalbumin, and differ markedly from the inflammatory vascular reactions in which fibrinoid deposits are observed and in which more or less acute necrotizing toxic influences prevail. Immune or allergic reactions play apparently a significant role in the production of both types of lesions and their underlying or associated plasmatic colloidal proteimic disturbances.

(b) *Amyloidosis*. The development of the proteimic amyloidosis not only is characterized by the presence of certain hematologic reactions (anemia, acceleration of erythrocytic sedimentation, increase in viscosity of serum) but is accompanied by more or less marked hyperproteinemia and especially hyperglobulinemia, which may become occasionally so excessive that the globulin portion of the plasma will separate from the nonglobulin part on standing (Bing, Eklund and Reimann, Reimann, Koucky and Eklund, Magnus-Levy, Morgenstern). This disease, which usually appears in man in connection with chronic suppurative conditions or disorders associated with protein wastage or with disturbances of protein metabolism (osteomyelitis, chronic tuberculosis, syphilis, lymphogranuloma, myeloma), leads to extracellular and intracellular deposition of a complex proteimic substance (amyloid) (Cohen, Danisch, Willer), especially in and around vascular and capillary walls (Hass and Schulz). As amyloidosis has been produced experimentally in animals by repeated and protracted parenteral introduction of certain foreign proteins—for example, in horses used for the production of antiserum (Arndt, Doerken, Reitstotter), rabbits, mice and others—or by feeding an unnatural proteimic diet (Jaffe, Grayzel, Jacobi, Marshall, Bogin and Bolker, Kuczinski, Ku and Simon, and others), amyloid has been related to excessive formation of a normal or an abnormal antigen-antibody complex (Letterer, Loeschke, Pringgaard, Lucke and Markley,

Koletsky and Stecher) or has been considered to be the result of a reaction of hypersensitivity.

Amyloidosis has been seen in man also in connection with chronic poisonings by lead, manganese and alcohol (Celli, Butt, Israel). It may be mentioned in this connection that the investigations of Ehrstrom have shown that a mixture of serum with chondroitin-sulfuric acid apparently precipitates albumin, causing thereby a relative increase of the serum globulin fraction. Murata and Yoshikawa observed that amyloid is deposited in rabbits given injections of, or fed, silicic acid, at sites where the silica accumulates in the tissues and undergoes a change from a sol into a gel, thereby showing that the physicochemical conditions ( $p_H$ ) of the tissue play a significant role in the distribution of amyloid (Morgenstern). Regardless of the primary or the secondary nature of amyloid, this proteimic matter is always deposited in the wall of the small and the medium-sized arteries of the various organs (heart, kidney, tongue and other organs) (Strauss, Binford, Koletsky and Stecher, Pearson, Rice and Dickens, Bell, Perla and Gross, Dillon and Evans). In some cases the aorta is also involved by medial nodular amyloid deposits. This proteimic condensation product, which usually contains 5 to 6 per cent of cholesterol, resembling therein the arteriolar subendothelial hyaline deposits with their lipoidal content, involves the subendothelial space and the media, where it spreads in between the muscle cells in homogeneous branching structures which ultimately merge into a solid mass under atrophy of the muscular elements (Peters). In large arteries the amyloid occupies in general the outer part of the media and the adventitia, whence it extends into the vascular wall along the amyloidotic vasa vasorum. The walls of the smaller vessels are highly thickened, and their lumens are usually considerably narrowed.

While M. B. Schmidt and also Leupold claimed that a precursor of amyloid circulates in the blood and penetrates from there into the vascular walls, Letterer suspected that the preamyloid substance is formed extravascularly and is precipitated in the vascular walls while passing through these tissues on its way to the blood.

Dick and Leiter observed in 12 per cent of rabbits given injections of various bacterial cultures medial necroses, calcifications and atheromatosis of the aorta in addition to widespread amyloidosis. This incidence represents, according to these investigators, six times the normal incidence of aortic lesions.

Hueper recently produced in dogs vascular lesions that were comparable in some respects by

injecting large amounts of solutions of foreign proteins, namely, gelatin and ovalbumin, intravenously over long periods. Dogs given solutions of ovalbumin or of an aminoantipyrine azocompound of ovalbumin had in the intima of the aorta and its branches hydropic mononuclear cell infiltrations, fibroblastic loose cushions and hyaline thickenings and in the media focal hyalinization and calcification. Some of the renal arterioles and small arteries exhibited thickened and locally hyalinized walls with proliferation of the endothelium.

The aortas of the dogs given solutions of gelatin, which elicits in the blood pseudoagglutination of the erythrocytes (Parkins, Koop, Riegel Vars and Lockwood) and behaves in some respects like globulin (Brunschwig, Scott, Corbin and Moe), displayed extensive edema and hyalinization of the intima with fine granular calcium deposits in the thickenings and in the internal elastic membranes, as well as large hyalinizations and marked edema of the media. The walls of the renal arteries and arterioles were vacuolated and edematous, and medial hyalinization and intimal hyaline thickenings were found in some instances (Hueper). Some of the arterioles were occluded by hyaline masses infiltrating the walls and containing irregularly arranged and shaped nuclei.

In this connection brief mention may be made of an observation recorded during the course of a toxicopathologic study of animals given injections of Evans blue (Hueper and Ichniowski). Eight molecules of this dye combine in the blood with one molecule of albumin, forming thereby a complex having the physicochemical characteristics of globulin (Rawson, Gregersen and Rawson). The dye is retained in the aortic wall and in other organs, particularly the testes, the teeth and the cartilage, over periods of more than six months. The aortas of dogs thus treated revealed small foci of intimal hyalinization, while the renal glomeruli of rabbits exhibited extensive hyalinizations. However, it is still uncertain whether or not the disturbance in the character of the serum albumins has any causal relation to these vascular manifestations. A similar binding of sulfonamide compounds to plasma albumins (Davis, Schonholzer) may be involved in the appearance of medial hyaline degenerations and calcifications of the aorta and the pulmonary and coronary arteries of rats following the administration of these chemicals (Endicott, Kornberg and Daft, Lehr, Antopol, Churg, and Spring, Daft, Ashburn, Spicer and Sebrell, Ashburn, Daft, Endicott and Sebrell). It is problematic whether or not extensive calcinotic degenerative

aortic lesions in mice painted with methylcholanthrene and fed a cystine-deficient diet are of similar genesis (White and Mider, White, Mider and Heston), or whether nutritional or vitamin deficiencies or metabolic disturbances play a causal role.

#### COLLOID PLASMATIC DISTURBANCES C. PROTEINIC TYPE

##### *Noninflammatory Vascular Reactions*

- Abrikosoff, A., and Wulff, F. *Verhandl d deutsch path Gesellsch* **22** 270, 1927
- Anitschkow, N. *Klin Wchnschr* **4** 2233, 1925
- Apitz, K. *Klin Wchnschr* **19** 1058, 1940, *Virchows Arch f path Anat* **306** 630, 1940
- Arndt, H. J. *Arch f Tierh* **63** 1, 1931, *Klin Wchnschr* **10** 910, 1931, *Verhandl d deutsch path Gesellsch* **26** 243, 1931
- Artificial Antibodies, editorial, *Science* **96** 181, 1942
- Ashburn, L. L., Daft, F. S., Endicott, K. M., and Sebrell, W. H. *Pub Health Rep* **57** 1883, 1942
- Askanazy, M. *Verhandl d deutsch path Gesellsch* **7** 32, 1904
- Bell, E. T. *Am J Path* **9** 185, 1933
- Beunhold, H. *Klin Wchnschr* **11** 2057, 1932
- Binford, C. H. *Arch Path* **29** 314, 1940
- Bing, J. *Acta med Scandinav* **103** 547, 1940
- von Bonsdorff, B., Groth, H., and Packalen, T. *Acta med Scandinav*, 1938, supp 89, p 347
- Brunschwig, A., Scott, V. B., Corbin, N., and Moe, R. *Proc Soc Exper Biol & Med* **52** 46, 1943
- Butt, E. M. *Arch Path* **10** 859, 1930
- Cantarow, A. *Am J M Sc* **189** 425, 1935
- Celli, P. *Sperimentale, Arch di biol* **89** 749, 1935 abstracted, *Zentralbl f allg Path u path Anat* **65** 388, 1936
- Chester, W. *Ztschr f klin Med* **124** 466, 1923
- Cohen, S. *Ann Int Med* **19** 990, 1943
- Cohn, E. J. *Chem Rev* **28** 395, 1941
- Daft, F. S., Ashburn, L. L., and Sebrell, W. H. *Science* **96** 321, 1942
- Ashburn, L. L., Spicer, S. S., and Sebrell, W. H. *Pub Health Rep* **57** 217, 1942
- Danisch. *Verhandl d deutsch path Gesellsch* **20** 307, 1925
- Davis, B. D. *Science* **95** 78, 1942
- Dick, G. F., and Leiter, L. *Am J Path* **17** 741, 1941
- Dietrich, A. *Verhandl d deutsch path Gesellsch* **21** 156, 1926
- Dillon, J. A., and Evans, L. R. *Ann Int Med* **17** 722, 1942
- Doerken, E. *Virchows Arch f path Anat* **286** 487, 1932
- Ehrstrom, M. C. *Acta med Scandinav* **101** 551, 1939
- Eklund, C. M., and Reimann, H. A. *Arch Path* **21** 1, 1936
- Endicott, K. M., Kornberg, A., and Daft, F. S. *Pub Health Rep* **59** 49, 1944
- Feller, A. E., and Fowler, W. M. *J Lab & Clin Med* **23** 369, 1938
- Foord, A. G., and Randall, L. *Am J Clin Path* **5** 532, 1935
- Grayzel, H. G., Jacobi, M., Marshall, H. B., Bogin, M., and Bolker, H. *Arch Path* **17** 50, 1934
- Gregersen, M. I., and Rawson, R. A. *Am J Physiol* **138** 698, 1943
- Gunn, F. D., and Mahle, A. E. *Arch Path* **26** 377, 1938

- Gutman, A B , Moore, D H , Gutman, E B , McClellan, V , and Kabat, E A J Clin Investigation **20** 765, 1941
- Hueper, W C Am J Path **18** 895, 1942
- and Ichmowski, C T Arch Surg **48** 17, 1944
- Israel, I Ein Fall von lokalem Amyloid, Inaug Dissert, Tubingen, Bochum-Langendreer, H Poppinghaus, 1933
- Jacobson, V C J Urol **1** 167, 1917
- Jegers, H , and Selesnick, S Internat Clin **47** 248, 1937
- Kabat, E A , Moore, D H , and Gutman, A B Paper presented at the meeting of the American Chemical Society, Buffalo, N Y, 1942
- Kagan, B M Am J M Sc **206** 309, 1943
- Kekwick, R A Biochem J **34** 1248, 1940
- Koletsky, S , and Stecher, R M Arch Path **27** 267, 1939
- Kracke, R R , and Hoffman, B J Ann Int Med **19** 673, 1943
- Ku, D Y , and Simon, M A Arch Path **18** 245 1934
- Letterer, E Beitr z path Anat u z allg Path **75** 486, 1926, Verhandl d deutsch path Gesellsch **20** 301, 1925
- Leupold, E Beitr z path Anat u z allg Path **64** 347, 1917-1918
- Loeschke, H Beitr z path Anat u z allg Path **77** 231, 1927
- Mahle, A E , Seed, L , and Welker, W H Arch Path **26** 441, 1938
- Melnick, D , Field, H , and Parnall, C G Arch Int Med **66** 295, 1940
- Morgenstern, Z Virchows Arch f path Anat **259** 698, 1926
- Muller, E Beitr z path Anat u z allg Path **97** 42, 1936
- Murata, M , and Yoshikawa, S Virchows Arch f path Anat **264** 587, 1927
- Parkins, W M , Koop, C E , Riegel, C , Vars, H M , and Lockwood, J S Ann Surg **118** 193, 1943
- Pauling, L J Am Chem Soc **62** 2643, 1940
- Pearson, B , Rice, M , and Dickens, K Arch Path **32** 1, 1941
- Perla, D , and Gross, H Am J Path **11** 93, 1935
- Perlzweig, W A , Delrue, G , and Geschickter, C J A M A **90** 755, 1929
- Peters, J T Arch Path **35** 832, 1943
- Randall, O S Am J Cancer **19** 838, 1933
- Rawson, R A Am J Physiol **138** 706, 1943
- Reimann, H A , Koucky, R F , and Eklund, C M Am J Path **11** 977, 1935
- Rosenblatt, M B Ann Int Med **8** 678, 1934
- Rosenblum, A H , and Kirshbaum, J D J A M A **106** 988, 1936
- Rosenheim, M L , and Wright, G P J Path & Bact **37** 332, 1933
- Schonholzer, G Klin Wchnschr **19** 790, 1940
- Shapiro, S , Ross, V , and Moore, D H J Clin Investigation **22** 137, 1943
- Shirer, J W , Duncan, W , and Haden, R L Arch Int Med **50** 829, 1932
- Strauss, A Virchows Arch f path Anat **291** 219, 1933
- Sweigert, C F Am J M Sc **190** 246, 1935
- Ulrich, H Arch Int Med **64** 994, 1939
- Vignati, J , and Rauchenberg, M Klin Wchnschr **16** 62, 1937
- Volland, W Virchows Arch f path Anat **298** 660, 1937
- White, J , and Mider, G B J Nat Cancer Inst **2** 95, 1941
- Mider, G B , and Heston, W E ibid **3** 453, 1943
- Willer, H Frankfurt Ztschr f Path **46** 306, 1933
- Wintrobe, M M , and Buell, M V Bull Johns Hopkins Hosp **52** 156, 1933

*Inflammatory Vascular Reactions*—(a) Allergy During the preceding discussions the occurrence of inflammatory, fibrinoid, necrotizing lesions affecting mainly the small and the medium-sized arteries has been repeatedly mentioned in connection with, and as complications of, vasotonic as well as hydrostatic arteriosclerotic changes. It was pointed out on these occasions that apparently superimposed allergic conditions are responsible for this type of reaction. In view of the fact that these manifestations are accompanied by an infiltration of the vascular wall by plasmatic material, giving rise to fibrinoid deposits, it appears likely that the inflammatory and necrotizing variety of vascular change occurs in association with plasmatic colloidal proteinic disturbances of allergic-hypereigic nature when the immune bodies formed during these processes possess toxic properties. The hyperproteinemia occurring during immune conditions is characterized by an increase in fibrinogen and globulin (Schittenhelm) and is associated with colloidoclastic leukopenia, thrombopenia, eosinophilia, a lengthened clotting time and an accelerated rate of sedimentation of the erythrocytes during acute crises of the immune state (allergic or anaphylactic shock). Such reactions are apparently related to the degree of dispersion of the interacting colloidal particles, as colloids consisting of highly dispersed small particles are less reactive than those with coarse particles (Klopstock).

Numerous observations connect allergic reactions with the development of degenerative arterial disease. Claims to this effect have been advanced repeatedly in regard to various vasospastic conditions (thromboangitis obliterans, angina pectoris, reactions suggesting Raynaud's disease, malignant hypertension) caused by endogenous or exogenous physical and chemical agents, such as foodstuffs, nicotine, pollen, drugs and cold (Vaughan, Davison, Thoroughman and Bowcock, van Creveld, Werley, Sullivan and Vaughan, Krauspe, von Eiselsberg, Gaensslen, Lichtwitz, Schmidt, Conti, Dattner, Kammerer). Joyner and Sabin reported that in certain allergic conditions the permeability of the capillary endothelium is reduced, preventing the removal of colloidal nontoxic dyes, while Klinge pointed out that anaphylactic shock can be prevented by the intravenous injection of colloidal



dyes as long as these remain in the colloidal state but not after they have precipitated in granular form. These observations on the involvement of colloidal phenomena in the production of allergic reactions are significant in view of the fibrinoid deposits found in the arterial subendothelial space in hyperergic reactions, where they may give rise to the formation of parietal thrombi, a complication especially important in the coronary arteries (Stenn, Horn and Finkelstein, Schlossmann).

The circle of the recognized or alleged allergic arterial diseases in recent years is rapidly widening, not only in the number of reported cases but also in the number of causative agents. Allergic arteritic changes occurring in connection with chronic infectious diseases, such as tuberculosis, streptococcic infections (Swift, Deick and Hitchcock, Siegmund, Strang and Semsroth, Semsroth and Koch, Karsner) and particularly rheumatic fever (Klinge, Junghanns, Masugi, Vaubel, Klinge and Vaubel, Abrikosoff, Grieshammer, Chiari, Coombs, Metz, Lieber, Von Glahn and Pappenheimer, Beneke, Abrikosoff and Rudik, Watjen, Klotz, Geipel, Schulz and Klinge, Gross Kugel and Epstein, Karsner and Bayless, von Sántha, Fossel, Pappenheimer and Von Glahn, Kugel and Epstein, McClenahan and Paul, Siegmund, Peila and Deutsch Gray and Aitken, Gegenbach) have figured prominently in this respect. Serum reactions and hypersensitivity responses to therapeutically administered sulfonamide compounds have entered more recently into this field (Clark and Kaplan, Rich, Longcope), while a third group is composed of diseases with causes evidently various or unknown but suspected to be of allergic nature, such as periarteritis nodosa, thromboangitis obliterans, isolated pulmonary sclerosis and temporal arteritis (Rossle, Jager). There are thus hyperergic vascular reactions conditioned by general sensitizing processes and those of local character, which, however, may be of general sensitizing nature but which are locally elicited by superimposed inflammatory processes (Abrikosoff).

The hyperergic reactions of the coronary and myocardial arteries and arterioles are associated with marked endothelial proliferations encroaching on the lumens, fibrinoid masses in the subendothelial spaces, considerable edema and displacement of the muscle by connective tissue in the media, histiocytic, lymphocytic and giant cell infiltration of the interstitial tissue and perivascular collars of histiocytes, plasma cells and lymphocytes (Junghanns, Klinge, Knepper and

Waalder, Vaubel, Masugi). Perivascular granulomas of the coronary vessels are found most frequently in the papillary muscle of the wall of the left ventricle and in the annulus fibrosus (Junghanns). Similar changes are encountered in the walls of the aorta, the pulmonary artery and its branches, and the renal, hepatic, cerebral and other large and small arteries (von Sántha, Stenn). Thrombi with subsequent organization and hemorrhages are seen in more acute conditions. The end result of such lesions is hyaline sclerosis or vascular obliteration by endarteritis. The chronic lesions in the stage of healing in which the inflammatory processes have subsided resemble closely those seen in other cicatricial types of arteriosclerotic changes (Chiari, Hanriot, Schmitt, Jager).

The hyperergic nature of the arterial reactions, resembling periarteritis nodosa, which were seen by Rich in 4 patients who had been treated with sulfonamide compounds, with or without injections of horse or rabbit immune serum is supported by observations recently made by French and Weller, who found in the hearts of 126 patients who came to autopsy after treatment with sulfonamide compounds eosinophilic leukocytic infiltrations of the myocardium together with myocarditis. Similar myocardial reactions could be elicited by French and Weller in mice given intraperitoneal injections of azosulfamide, sulfanilamide, sodium sulfapyridine and sodium sulfathiazole. Rich and Gregory reproduced in rabbits arteritic lesions of the periarteritis nodosa type involving the heart, liver, kidneys, adrenal glands, testes, lungs, spleen and pancreas by injecting horse serum and sulfadiazine. Similar changes were observed by Clark and Kaplan in 2 patients who died from serum sickness after an injection of horse serum. Arteritic lesions of the same type (perivascular granuloma, fibrinoid swelling of the wall, necrosis, cellular infiltration, endothelial proliferation) were produced in animals by injections of foreign protein by numerous investigators (Rich and Gregory, Vaubel, Aplitz, Junghanns, Takeda, Masugi, Sato and Todo, Ceroli, Heinlein, Heinlein and Muschalik, Rintelen, Migounov, Knepper and Waaler, Fox and Jones, Stecher). Variations in extent and degree in the reactions observed by the different investigators may be related to the fact that serums of different species were used in different animals. Longcope called attention to the fact that normal and immune serums differ in antigenic properties depending on species. Practically identical inflammatory necrotizing arterial reactions were elicited in animals by repeated injections of bacterial cultures or vaccines,



especially of streptococci (Metz, Masugi and Isibasi)

(b) *Periarteritis Nodosa* The incidence of periarteritis nodosa has undergone in recent decades considerable changes. While the majority of cases reported during the past century were reported individually, larger series of cases have been placed on record in the last four decades (von Baló). Von Baló and Nachtnebel noted in 1929 that the majority of cases on record were reported from Germany (Gruber, Jager, Monckeberg, Gohrbrandt, Wohlwill, Bierner, Kimmelstiel), with small numbers of cases being published in England, the United States, Australia and a few of the smaller countries. While Germany still seems to occupy the leading position, an astonishingly large number of cases have been reported from this country during recent years, of which only a few are listed here (Lund, Fitz, Parks and Branch, Malamud and Foster, Berger and Weitz, Davidsohn, Banowitch, Polayes and Charet, Allen, Coe, Reisman and DeHoff, Blaisdell and Pointer, McCall and Pennock, Motley, Felsen, Lebowich and Hunt, Jones, Weit, Wever and Perry, Haining and Kimball, Harris, Lynch and O'Hare). Jones collected in 1939 a total of 101 cases recorded in the English literature.

The disease affects persons of all ages and small and large vessels, irrespective of the presence of vasa vasorum (Lange). Of a total of about 230 patients whose cases were reported up to 1937, 32 (13.9 per cent) were children (Coe, Reisman and DeHoff). The mesenteric (Haining and Kimball) and renal vessels were apparently most often affected (80 per cent of the cases, Arkin, 100 per cent, McCall and Pennock), and nephrosclerosis, therefore, often accompanied the disease. *Periarteritis nodosa* may affect the vessels of individual organs only—for example, pulmonary vessels (Steinberg, Rossle) or renal vessels (Hauser)—or may involve practically the entire arterial and sometimes also the venous system. In view of the frequently acute character of the arteritic processes multiple aneurysms are often present. The disease, which often appears after acute infections, usually takes a relatively rapid and fatal course, but some patients survive for several years, and occasional ones are cured. The disease occurs also in animals—cattle (Hoogland, Guldner, Nieberle), hogs (Joest, Joest and Harzer, Hoogland, Henschen, Nieberle), deer (Lupke, Jager) and dogs (von Baló). Eosinophilia of the blood is often observed and is sometimes marked.

Attempts aimed at the isolation of specific causative agents from the tissues and the blood

of affected persons have failed (Gohrbrandt, von Hann). The claim made by von Hann as to the experimental reproduction of the disease in guinea pigs given injections of blood from a patient is based on a misinterpretation of normal conditions in the lungs of guinea pigs (Lemke). Selye and Pentz recently advanced the theory that periarteritis nodosa, rheumatic arteritis and nephrosclerosis may be causally related in part to abnormal and probably excessive adaptive responses of the adrenal cortex.

(c) *Temporal Arteritis* It is likely that the giant cell chronic arteritis of the temporal artery belongs to this complex of allergic arterial reactions. Chasnoff and Vorzimek considered it a local manifestation of a systemic disease usually ending after a number of months in recovery but occasionally causing death. The lesions in the temporal arteries differ histologically sufficiently from those seen in periarteritis nodosa and rheumatic arteritis to make them a distinct entity. The changes, however, are not limited to the temporal arteries but occur also in the carotid, central retinal, cerebral, occipital and radial arteries. Similar giant-cellular necrotizing granulomatous medial changes affecting the aorta, the branches of the aorta and their branches have been seen by Gilmour in 4 cases. Spioul and Hawthorne reported similar lesions in the aorta and the iliac arteries of 2 patients. Hoyt, Perera and Kauvar and also Weiss recorded cases of temporal arteritis and the changes considered as common local manifestations of a generalized arterial disease. Additional reports dealing with temporal arteritis have been made (Horton, Magath and Brown, MacDonald and Moser, Bowers, Dick and Freeman, Jennings, Bain, Horton and Magath, Sprague and MacKenzie), making a total of 21 cases. The disease usually involves elderly persons, more often those of the female sex. The lumens of the rigid vessels are narrowed or obliterated by proliferation of the intima. The media is invaded by monocytes and replaced in parts by a granulomatous tissue containing giant cells. Thrombi occur occasionally. Aneurysmal sacs may arise in the media.

#### COLLOID PLASMATIC DISTURBANCES C. PROTEINIC TYPE

##### *Inflammatory Vascular Reactions*

- Abrikosoff, A. I. *Virchows Arch f path Anat* **295** 669, 1935  
 —and Rudik, D. *Arch internat de med exper* **10** 303, 1935  
 Allen, P. D. *Arch Surg* **40** 271, 1940  
 Apitz, K. *Virchows Arch f path Anat* **289** 46, 1933  
 Bain, C. W. C. *Lancet* **1** 517, 1938  
 von Baló, J. *Virchows Arch f path Anat* **248** 337, 1924

- and Nachtnebel, E *ibid* **272** 478, 1929
- Banowitch, M M, Polayes, S H, and Charet, R  
Ann Int Med **16** 1149, 1942
- Beneke, R Virchows Arch f path Anat **254** 723, 1924
- Berger, S S, and Weitz, M A J Allergy **9** 489, 1938
- Blaisdell, E R, and Porter, J E New England J Med **224** 1087, 1941
- Bowers, J M Arch Int Med **66** 384, 1940
- Brenner, F Frankfurt Ztschr f Path **51** 479, 1938
- Chasnoff, J, and Vorzimer, J J Ann Int Med **20** 327, 1944
- Charr, H Beitr z path Anat u z allg Path **80** 336, 1928, **88** 1, 1931, Klin Wchnschr **9** 1862, 1930
- Clark, E and Kaplan, B I Arch Path **24** 458, 1937
- Coe, M Reisman, H A and DeHoff, J J Pediat **18** 793, 1941
- Conti Atti Soc lomb sc med e biol **18** 121, 1929
- Coombs, C Quart J Med **2** 26, 1908
- van Creveld, S Ann paediat **157** 84, 1941
- Dattner, B Nervenarzt **4** 573, 1931
- Davidsohn, I Illinois M J **83** 352, 1943
- Davison, H M, Thoroughman, J C, and Bowcock, H South M J **36** 560, 1943
- Dick, G F, and Freeman, G J A M A **114** 645, 1940
- von Eiselsberg, K P Klin Wchnschr **12** 1174 1933, **13** 619, 1934, Wien klin Wchnschr **45** 332, 1932
- Felsen, J Ann Int Med **15** 251, 1941
- Fitz, R, Parks, H, and Branch, C F Arch Int Med **64** 1133, 1939
- Fossel, M Frankfurt Ztschr f Path **54** 588, 1940
- Fox, R A, and Jones, L R Proc Soc Exper Biol & Med **55** 294, 1944
- French, A J, and Wellet, C V Am J Path **18** 109, 1941
- Gegenbach, A Zentralbl f allg Path u path Anat **36** 244, 1925
- Geipel, P Munchen med Wchnschr **54** 1057, 1907, **56** 2469, 1909, Deutsches Arch f klin Med **85** 75 1905
- Gilmour, J R J Path & Bact **53** 263, 1941
- Gohrbrandt, P Virchows Arch f path Anat **263** 246, 1927
- Gray, S H, and Aitken, L Arch Path **8** 451, 1929
- Grieshammer, W Klin Wchnschr **18** 451, 1939, Frankfurt Ztschr f Path **53** 136, 1939
- Gross, L, Kugel, M A, and Epstein, E Z Am J Path **11** 253, 1935
- Gruber, G B Virchows Arch f path Anat **245** 123, 1923, **248** 441, 1925, Verhandl d deutsch path Gesellsch **19** 313, 1923
- Haining, R B, and Kimball, T S Am J Path **10** 349, 1934
- von Hann, F Virchows Arch f path Anat **227** 90, 1919
- Harris, A W, Lynch, G W, and O'Hare, J P Arch Int Med **63** 1163, 1939
- Hemlein, H Arch f exper Path u Pharmakol **179** 127, 1935, Virchows Arch f path Anat **299** 667, 1937
- and Muschalik Klin Wchnschr **16** 873, 1937
- Horton, B T, and Magath, T B Proc Staff Meet, Mayo Clin **12** 548, 1937
- Magath, T B, and Brown, G E Arch Int Med **53** 400, 1934
- Hoyt, L H, Perera, G A, and Kauvar, A J New England J Med **225** 283, 1941
- Jacger, A Verhandl d deutsch path Gesellsch **13** 209, 1909, Virchows Arch f path Anat **197** 71, 1909
- Jager, E Virchows Arch f path Anat **284** 526, 1932, **288** 833, 1933
- Jennings, G H Lancet **1** 424, 1938
- Joest, E, and Harzer, J Beitr z path Anat u z allg Path **69** 85, 1921
- Jones, G M Ann Int Med **16** 920, 1942
- Joyner, A C, and Sabim, F R J Exper Med **68** 325, 1938
- Junglinns, E Beitr z path Anat u z allg Path **92** 467, 1933
- Kammerer, H Allergische Diathese und allergische Erkrankungen, Munich, J F Bergmann, 1934, p 271
- Karsner, H T Am J Path **10** 704, 1934
- and Bayless, F Am Heart J **9** 551, 1934
- Kimmelstiel, P Virchows Arch f path Anat **265** 16 1925
- Klinge, F Klin Wchnschr **6** 2265, 1927, **9** 586, 1930, Beitr z path Anat u z allg Path **83** 185, 1930, Virchows Arch f path Anat **278** 438, 1930, **279** 1, 1930
- and Vaubel, E *ibid* **281** 701, 1931
- Klinger, H Frankfurt Ztschr f Path **42** 455, 1931
- Klopstock, A Immunität in Lichtwitz, L, Liesegang, R E, and Spiro, K Medizinische Kolloidlehre, Leipzig, Theodor Steinkopff, 1935, p 193
- Knepper, R, and Waaler, G Virchows Arch f path Anat **294** 587 1935
- Krauspe Klin Wchnschr **15** 1742, 1936
- Kugel, M A, and Epstein, E Z Arch Path **6** 247, 1928
- Lange, F Virchows Arch f path Anat **248** 463, 1924
- Lebowich, J, and Hunt H D Am J Clin Path **10** 542, 1940
- Lemke, R Virchows Arch f path Anat **245** 322, 1923
- Lichtwitz Klin Wchnschr **4** 2353, 1925
- Lieber, M M Beitr z path Anat u z allg Path **91** 594, 1933
- Longcope, W T Medicine **22** 251, 1943
- Lund, H Z Ohio State M J **38** 244, 1942
- McCall, M and Pennock, J W Am J M Sc **206** 652, 1943
- McClenahan, W U, and Paul, J R Arch Path **8** 595, 1929
- MacDonald, J A, and Moser, R H Ann Int Med **10** 1721, 1937
- Malamud, N, and Foster, D B Arch Neurol & Psychiat **47** 828, 1942
- Masugi, M Beitr z path Anat u z allg Path **91** 82, 1933, Klin Wchnschr **14** 373, 1935
- and Isibasi, T Beitr z path Anat u z allg Path **96** 391, 1936
- and Ya-Shu Virchows Arch f path Anat **302** 39, 1938
- Sato, Y, and Todo, S Tr Soc path jap **25** 211, 1935
- Metz, W Beitr z path Anat u z allg Path **88** 17, 1931
- Migounov, B I Acta rheumat **6** 9, 1934
- Monckeberg, J G Beitr z path Anat u z allg Path **38** 101, 1905
- Motley, L J A M A **106** 898, 1936
- Nieberle, K Virchows Arch f path Anat **256** 131, 1925, **269** 587, 1928
- Pappenheimer, A M, and Von Glahn, W C Am J Path **3** 583, 1927, **2** 15, 1926, J M Research **44** 489, 1924

- Perla, D, and Deutsch, M *Am J Path* **5** 45, 1929
- Rich, A R *Bull Johns Hopkins Hosp* **71** 123, 1942  
—and Gregory, J E *ibid* **72** 65, 1943
- Rintelen, W *Virchows Arch f path Anat* **299** 629, 1937
- Rossle, R *Virchows Arch f path Anat* **288** 780, 1933, *Klin Wchnschr* **12** 574, 1933
- von Santha, K *Virchows Arch f path Anat* **287** 405, 1932
- Schittenhelm, A, in Mohr, L, and Staehelin, R *Handbuch der inneren Medizin*, ed 2, Berlin, Julius Springer, 1925, vol 1, p 1
- Schlossmann, N C *Arch Path* **34** 365, 1942
- Schmidt, R *Munchen med Wchnschr* **77** 1435 1930
- Schmitt, H *Virchows Arch f path Anat* **296** 603 1936
- Schulz, M, and Klinge, F *Virchows Arch f path Anat* **288** 717, 1933
- Selye H, and Pentz, E I *Canad M A J* **49** 264, 1943
- Semsroth, K, and Koch R *Krankheitsforsch* **8** 191 1930
- Siegmund, H *Zentralbl f allg Path u path Anat* **32** 276, 1924 **51** 385 1931, *Verhandl d deutsch path Gesellsch* **19** 114, 1923 *Ztschr f Kreislauforsch* **21** 389, 1929
- Sprague P H and Mackenzie W C *Canad M A J* **43** 562, 1940
- Sproul, E E, and Hawthorne, J J *Am J Path* **13** 311, 1937
- Stecher W *Virchows Arch f path Anat* **300** 645 1937
- Stenn F *Arch Path* **26** 244, 1938
- Strang J M, and Semsroth, K *Arch Int Med* **47** 583, 1931
- Sullivan, C I, and Vaughan W T *J Allergy* **9** 48, 1937
- Swift H F, Derick C L and Hitchcock, C H *J A M A* **90** 906, 1928
- Takeda K *Tr Soc path jap* **25** 194, 1935
- Vaubel E *Beitr z path Anat u z allg Path* **89** 374, 1932, **83** 185, 1929
- Vaughan, W T *The Practice of Allergy*, St Louis, C V Mosby Company, 1939, p 1014
- Von Glahn, W C, and Pappenheimer, A M *Am J Path* **2** 235, 1926
- Watjen *Verhandl d deutsch path Gesellsch* **18** 223, 1921
- Weiss S *New England J Med* **225** 579, 1941
- Werley G *J Allergy* **4** 65, 1932, *Southwest Med* **22** 431, 1938
- Wever, G K, and Perry, I H *J A M A* **104** 1390, 1935
- Wevi, D R *Am J Path* **15** 79, 1939
- Wohlwill, F *Virchows Arch f path Anat* **246** 377, 1923

#### HEMATIC ANOXEMIA

Agents that cause the production of inert hemoglobin derivatives, such as methemoglobin, carbon monoxide-hemoglobin and sulfmethemoglobin, which lack the oxygen-carrying power of normal hemoglobin interfere with the proper oxygenation of the vascular wall and may thereby give rise to the development of degenerative changes in the vascular wall. Inasmuch as many

of these agents exert also a vasotonic effect, then arteriosclerogenic action is based on the summation of two different causative mechanisms. Agents of this type are carbon monoxide, nitrites and sulfonamide compounds. It is likely that the medial necrosis in the aortas of rabbits produced by Loewe, Jurgens and Noltemeier by the intravenous injection of chloramine and by Mancke and Droop by the oral administration of formaldehyde or formaldehyde sodium bisulfite is in part a result of the formation of methemoglobin. A similar mechanism was probably active in the production of medial necroses in the arteries of rabbits following the intravenous injection of chloropicrin and benzoyl peroxide (Mullei) and of hydrogen peroxide, magnesium peroxide, benzoyl peroxide, acetylchloroammonium benzol hydroquinone, quinone and methylthionine chloride (Rieder, Siebert), and after the percutaneous application of toluene sulfodichloroamide, dichloroamidobenzoic acid acetylchloroaminobenzol and chloroamido carbonic ethyl ester.

A similar hematic effect on the oxygenation of the vascular walls occurs if the blood is not completely saturated with oxygen while passing through the lungs because of reduced atmospheric oxygen pressure. Campbell, as mentioned before, reported that animals kept for several weeks in an atmosphere with greatly reduced oxygen tension showed intimal thickening of their pulmonary arterioles. However, a greatly increased oxygen pressure of the atmospheric air also interferes with adequate oxygenation of the tissues by causing an imbalance between the carbon dioxide and the oxygen tension in the tissues and an accumulation of carbon dioxide in the tissues and by eliciting thereby hyperoxic hypoxidoses (Strughold, Gsell, Bean and Bohr, Hinshaw and Boothby). This mechanism is in part responsible for the intimal thickenings of the pulmonary arteries observed in rats subjected for more than twenty-four days to inhalation of compressed air with an oxygen tension of 635 mm of mercury (Bennett), and such disturbances in the carbon dioxide-oxygen balance of the blood and tissues are associated also with vasculotonic reactions which accentuate the hematic anoxic arteriosclerogenic effects. These anoxic reactions are, moreover, responsible for the development of myocardial degenerations in oxygen poisoning (Kaunitz).

#### HEMATIC ANOXEMIA

- Droop, H *Experimentale Untersuchungen an Kaninchen über die Stoffwechselgenese der Arteriosklerose und die diätetische Beeinflussung dieses Prozesses*. Inaug Dissert, Göttingen, W F Kaestner, 1915
- Gsell R *Am J Physiol* **66** 5, 1923

- Hinshaw, H. C., and Boothby, W. M. *Proc Staff Meet, Mayo Clin* **16** 211, 1941
- Kaunitz, J. *J Aviation Med* **13** 267, 1942
- Loeb, O. *Arch f exper Path u Pharmacol* **69** 114, 1912
- Mancke, R. *Arch f exper Path u Pharmacol* **141** 228, 1929
- Muller, W. *Weitere Beitrage zur Kenntnis der resorptiven Wirkung der Oxydationsmittel*, Inaug Dissert, Gottingen, E. Hofer, 1919
- Noltmeier, H. H. T. *Beitrage zur experimentellen Pharmakologie des disponiblen chlores*, Inaug Dissert, Gottingen, E. Hofer, 1919
- Rieder. *Ztschr f d ges exper Med* **10** 169, 1920
- Rossmann's. *Inaug Dissert*, Gottingen, 1920
- Siebert. *Ztschr f d ges exper Med* **9** 123, 1919
- Wenzel, H. *Arch f exper Path u Pharmacol* **137** 215, 1928

## PREVENTIVE AND THERAPEUTIC ASPECTS

In his recent treatise on vascular sclerosis Moschcowitz came to the conclusion that

unless the individual is cut down by intercurrent disease, death is invariably the result of arteriosclerosis. It is the inevitable destiny of all creatures who possess a cardiovascular system with intravascular pressure.

Arteriosclerosis being an inevitable consequent of ageing and therefore an irreversible process, it is hardly likely that any method of therapy will ever be discovered which will restore the diseased vessels to their normal texture, unless we can cure mortality. Nor can arteriosclerosis be prevented, no more than gray hair or facial wrinkles.

This fatalistic attitude based on the unrestricted acceptance of the old age theory of arteriosclerosis finds its equivalent in and has as much merit as, the one propounded by the proponents of the old age theory of cancer, who are equally emphatic in their dictum that cancer is the ultimate cause of death of all persons living sufficiently long. It was pointed out recently by Hueper that there exists a sufficient amount of reliable and valid evidence showing that cancer is not an obligatory outcome of senescent tissue changes but is a reaction to abnormal exogenous and endogenous factors of physical and chemical nature. It must be equally clear from the experimental and clinical data presented here that also arteriosclerosis in its various types is essentially not an old age disease but a condition brought about by various exogenous and endogenous agents and influences acting on the blood and the vessels during the major part of life and independent of any senescence process. Cancer and arteriosclerosis are definite disease phenomena and not physiologic or pathologic manifestations of old age. They are for this reason amenable to preventive and therapeutic measures. The fact that the means at present at one's disposal are defective and in part irrational does not detract from the validity of the foregoing statement but should provide

inspiration for the development of improved measures.

It is felt that the preceding presentation dealing with the numerous causes and the different causative mechanisms and their related anatomic vascular manifestations furnishes an adequate basis for the development of rational diagnostic, preventive and therapeutic methods. The evidence presented, however, definitely indicates that there is no single approach to this problem which will be equally effective for the preventive and the therapeutic management and the diagnosis of the different types of degenerative and sclerosing vascular lesions.

The diagnostic methods and the therapeutic or preventive measures needed for the demonstration and the treatment of the hematic changes underlying and preceding the development of the vascular reactions caused by colloidal plasmatic disturbances will be different from those indicated for the discovery and the therapy of the functional and the anatomic vascular and organic reactions associated with arteriosclerosis on the basis of vasotonic and hydrostatic factors. In planning the control of these vascular diseases consideration must be given to the facts that arteriosclerosis is a disease complex that starts its anatomic development during middle adult life and that all therapeutic measures are utterly incapable of restoring an arterial system with cicatricial arteriosclerotic lesions in which the functioning elastic and muscular elements of the vascular wall are replaced by nonfunctioning fibrous, hyaline and calcified matter to its original state of functional and anatomic integrity. Only in the early stages of the disease can it be cured while in advanced stages merely its further progress can be arrested or impeded (Plesch). It is pertinent, therefore, that any preventive measures must be started at a time when there are only functional vascular or hematic changes present, i. e., during the early part of adult life. It should be equally clear that effective diagnostic supervision and preventive management of the patient should from then on extend over the remaining part of life if sickness, disability and death from arteriosclerotic disease is to be avoided or to be appreciably limited.

While it is beyond the scope of this presentation to deal in detail with the prevention and the therapy of arteriosclerosis it seems advisable to indicate the general lines of approach by which this goal might be achieved as they appear from this investigation. Measures directed at the prevention of the deposition of lipid material in the arteries must aim at keeping the plasma cholesterol level within the lower part of the normal

range of, if that is not possible because of some uncontrollable factor, at improving the stability of the colloidal dispersed cholesterol so as to prevent its precipitation in and on the vascular walls. The reduction of the cholesterol level of the plasma may be attained by limiting the alimentary intake of cholesterol-containing foodstuffs (Tuohy, Leary) and by administering blood cholesterol-lowering agents, such as, especially, thyroid preparations and, in diabetic conditions, possibly, lipocain in conjunction with insulin. The stabilization of the cholesterol sol in the plasma at a safe level may be accomplished by the oral introduction or the parenteral injection of peptizing agents which increase the colloidal dispersion of the cholesterol and thereby lower its precipitability. It is possible that the favorable effect seen from the use of thiocyanides and iodine compounds of fatty acids (diiodine ricinoleate) in experimental cholesterol atheromatosis depends on this mechanism. Consideration should be given in this respect also to the lecithin and bile acid content of the plasma. The more difficult problem of a rapid and effective mobilization of lipid material in the atheromas and its removal with the blood, which is especially important in atheromatosis of the coronary arteries, may perhaps be approached by applying *in vivo* the methods used in ordinary life for the removal of greasy material from fabrics through the employment of colloidal emulsifiers and detergent solubilizers. However, it should be emphasized in this connection that a great deal more information on the factors controlling the colloidal equilibrium of the plasma is necessary before real progress in this field will be likely.

Recent experiences with the experimental production of vascular diseases by changes in the plasma proteins direct attention to certain so far neglected aspects of modern chemotherapy with sulfonamide compounds and related compounds. It seems to be necessary that more attention be paid to the possibility of allergic vascular complications arising from such procedures. The observations made on such occasions suggest, moreover, that vitaminic imbalances resulting from such therapy and apparently affecting mainly the vitamin B complex require closer study as to their arteriosclerogenic potentialities.

The occurrence of degenerative and cystic medial lesions in the aorta as late results of severe hypotonic episodes make it urgent that conditions of circulatory failure, such as those seen in shock, are cut as short as possible with the existing therapeutic measures. Persons who have passed through prolonged and marked hypotensive states should be cautioned against any ex-

cessive strain to their vascular system, particularly during the early period following such an attack, i.e., before firm scar tissue may have formed.

The prevention of chronic arterial lesions on the basis of occupational or environmental exposure to vasotonic chemicals with hypotonic (carbon monoxide, nitrites, cyanides) or hypertonic effect (digitalis, viosterol, ephedrine and derivatives, nicotine, lead) is mainly a matter of industrial and public hygiene and thus a subject of legislation and education.

It is obvious that adequate preventive measures cannot always be instituted in time, because the exposure to the etiologic vasotonic agents is not properly recognized or cannot be combated for various technical reasons or is disregarded out of negligence. The development of the ultimately appearing degenerative and sclerosing arterial lesions raises then the question of the use of appropriate therapeutic measures. The type of therapeutic measure adopted depends on the character of the causative arteriosclerogenic vasotonic mechanism present and on the kind of therapeutic action desired or possible in the individual case. Three different types of therapeutically active agents may be chosen for this purpose if available.

- 1 Agents which destroy the vasotoxic principle by oxidation, reduction, hydrolysis, conjugation or other types of metabolic disintegration or alteration or which make the vasotoxic principle innocuous and ineffective by blocking its site of action (structural blocking)

- 2 Agents which eliminate or increase the excretion of a causative vasotonic principle. The administration of calcium salts, viosterol and parathyroid hormone in chronic lead poisoning or the removal of parathyroid adenomas in hyperparathyroidism belong in this category.

- 3 Agents which exert a vasotonic effect opposite to that exerted by the causative principle. This type of therapeutic measure is obviously the least desirable and effective one as it usually merely produces symptomatic relief without influencing in any way the continued existence of the causal conditions. Vasodilating agents, such as nitrites, thiocyanides, theobromine, acetylcholine and similarly acting substances, are used in the symptomatic control of hypertension of various genesis, while epinephrine, ephedrine, extract of adrenal cortex and other hypertonic agents are employed in counteracting hypotensive states of diverse nature. Inasmuch as the site of action of a vasculotonic agent depends on its type and on

the dose administered, it is advisable to select for therapeutic purposes a vasotonic agent which exerts its effect in the same region in which the causal arteriosclerogenic vasotonic agent is operative. Disregard of this principle may result in apparent symptomatic relief through the production of abnormal functional and, finally, anatomic changes in some other part of the cardiovascular system. To lower increased blood pressure caused by vascular hypertonia through interference with cardiac function resulting in a reduction of the cardiac output is thus not only an irrational but a harmful procedure.

It is evident from these considerations that the available therapeutic measures are of only limited

value, as at best they do no more than prevent any further progress of the arteriosclerotic process. In general they restrict to some extent the unhampered advance of the vascular changes or simply produce some temporary symptomatic relief without interfering seriously with the development of these changes.

#### REFERENCES FOR PREVENTIVE AND THERAPEUTIC ASPECTS

- Hueper, W. C. *Occupational Tumors and Allied Diseases*, Springfield, Ill., Charles C. Thomas, Publisher, 1942.  
 Moschcowitz, E. *Vascular Sclerosis*, New York, Oxford University Press, 1942, p. 178.  
 Plesch, Klm. Wchnschr **11** 524, 1932.  
 Tuohy, E. L. I. A. M. A. **121** 42, 1942.

## Book Reviews

**The Biological Basis of Individuality** By Leo Loeb professor emeritus of pathology, Washington University School of Medicine, St Louis Price \$10.50 Pp 711 Springfield, Ill Charles C Thomas, Publisher 1944

The comprehensiveness of this book makes it difficult to review all parts of it with equal adequacy, an attempt, therefore is made to convey to the reader of this review only the main gist of the book in which the author has synthesized the results of his own studies and those of related investigations by other biologists into a highly original and far reaching system

Individuality is the original physical and psychic state of an organism which has developed in accordance with its genetic constitution in cooperation with environmental factors. The concept of a biologic basis of individuality originated in the well known investigations on transplantation which the author has conducted during the past five decades. Transplantation of tissue from one animal to another calls forth a cellular reaction which is the stronger the greater the strangeness between host and donor. Only autogenous grafts fail to elicit such a reaction, but even if host and donor are as closely related as litter mates of a strain of mice inbred through many generations, the transplants will produce a mild reaction in the host. The results of the latter syngenesiotransplantations indicate incidentally that complete homozygosity has as yet not been achieved by continuous inbreeding.

There are indications that the reaction around a graft is caused by certain proteins, probably by nucleoproteins given off by the transplant. Each individual is the carrier of certain proteins or of a certain protein which distinguishes all the organs and tissues of this individual from the organs and tissues of any other individual of the same species and strain. This distinctive substance, termed "individuality differential," is present in all tissues and organs and also in the blood of an individual. Besides this individuality differential, each organism carries a number of substances typical of the strain, the species or the class to which it belongs and which distinguish it from the members of all other strains, species or classes. All these distinctive substances, including the individuality differential, are designated as "organismal differentials."

Species differentials are responsible for the marked reaction that a heterotransplant elicits in the host leading to quick destruction of the graft. The violence of this reaction makes heterotransplantation less suitable for the detection of species differences than serologic methods which demonstrate the presence of antibodies in the serum of immunized animals. For the detection of finer differences between individuals, transplantation is not only superior to serologic methods but the method of choice, although individuality differentials have been shown to exist in the red corpuscles of certain animals. In addition, there are the organ and tissue differentials. They are responsible for the difference in the reactions elicited in the host by various tissues of the same individual.

Data on the responses of the host to various types of transplants and on the relation of the differentials

to varying conditions in the host (age, hormonal influences, blood groups) occupy almost one fourth of the book and include much new material. In conclusion two distinct types of biologic individuality are recognized. 1 The "mosaic individuality" based on the sum of the organ and tissue differentials carried by an individual. This is the individuality recognized so far and studied in particular by the geneticists. 2 The "essential individuality" based on the distinction between the individuality differential of one individual from the individuality differentials of all other individuals. Both mosaic and essential individuality depend primarily on genetic factors, but they are also under the influence of environmental conditions.

Subsequently, the phylogenetic and the ontogenetic development of the differentials is traced from lower organisms, such as coelenterates, planaria and amphibians to birds and mammals. In the course of evolution of animals a finer differentiation takes place and a more rigid specificity of the differentials ensues. Thus in primitive species heterotransplantation even of whole organs may succeed, and two individuals may be joined together without development of signs of incompatibility such as occurs, for instance, in parabiotically joined rats. Corresponding to the phylogenetic evolution of the differentials there is a progressive ontogenetic development of the latter. Against embryonic tissue reactions may be lacking or slight, and tissues of young individuals produce milder reactions in the host than those of adults. Thus, in young organisms the proteins representing the individuality differentials have not as yet achieved as high a specificity as in the adult. There is, therefore, an inverse relation between the adaptability of an organism and the rigidity of its individuality. A certain relation exists also between the development of the organismal differentials and the organizers. With advancing embryonal development the organizers are replaced by complex highly specific contact substances which maintain the normal equilibrium between neighboring tissues and organs. Graded manifestations of compatibility or incompatibility between unicellular organisms suggest that even in these primitive structures there may exist finely differentiated substances analogous to the organismal differentials of higher species. However, these substances are not identical with true organismal differentials. From this point of view the author analyzes the reactions taking place between individual protozoa and those between spermatozoon and egg, he also discusses the interaction between single cells during the processes of tissue formation from the primitive amoebocytes of *Limulus* to the cells of higher organisms.

Transplantation as a systematic method for tumor research was first used by the author in 1901. Tumors are bearers of the same or almost the same organismal and individuality differentials as the normal organs and tissues of the individual in which they originated. The better transplantability of tumors, i.e., the successful transplantation into homozygous and even heterozygous animals is due largely to the increased growth energy of the tumor and to processes of adaptation between host and donor. These processes are fully



discussed with particular reference to the production of immunity to transplanted tumors. The author holds the view that the development of a spontaneous tumor is not due to a somatic mutation. This interpretation agrees with the conclusion that factors underlying transplantability of tumors are not the same as those involved in spontaneous tumor growth.

The following part of the book deals with the nature of the organ and tissue differentials and with their mutual relations, and also with the relations between organ and organismal differentials. It has as yet not been possible to isolate these antigenic differentials by chemical means. They are, however, not identical with any of the known antigens which have so far been analyzed in studies on blood groups, Rh factor, precipitins, hemolysins or cytotoxins. They are genetically determined but are not present in the genes, the latter contain or may determine the formation of precursor substances which during ontogenesis develop into the differentials proper.

The presence of the same individuality differential in all organs and tissues of an individual makes possible the normal interaction between the various tissues and between tissues and body fluids. In order to interact harmoniously, the various tissues and body fluids which represent the inner environment of the body have to be specifically adapted to one another. Thus there is in the healthy organism an "autogenous equilibrium" meaning identity of the individuality differentials in tissues and body fluids of the same individual. Disharmonies in this balance give rise to disease. Similarly, aging represents a continuous decline of the mutual adaptation between the constituent parts of an organism, ultimately leading to death. The lower the species, the fewer and less complex are the tissues and substances involved in the maintenance of a normal equilibrium. In lower organisms not only entire organs but even a whole new organism may be regenerated from parts of the old one after injury. These lower organisms are thus potentially immortal. Higher organisms have developed such a complex system of structure and function that they have lost their adaptability to unfavorable environmental conditions. They have become rigid and have acquired senescence and disease, they have attained refinement of individuality but at the expense of immortality.

While thus in the course of evolution the higher organisms have lost to a large extent their power of adaptation to the outer environment, the central nervous system and higher sense organs which serve as a means of communication between the individual and his environment have developed a high specialization and extended their significance. In the sphere of the psychic aspects of the individuality, the organism has become more dependent on the environment. These considerations lead to the final chapter of the book dealing with the psychosocial aspect of individuality. The psychic attitude of an individual is in the last analysis determined by reactions of his nervous system which in turn is also a carrier of his individuality differential. This being genetically determined, all actions and emotions of an individual are likewise under the influence of his genetic constitution. However, environmental conditions in higher organisms play a prominent role in determining psychic and social attitudes. This is in contrast to the physical makeup of the individual which is preponderantly influenced by genetic factors and only to a lesser extent by environmental conditions.

As with many a masterwork on biology, objections may possibly be raised by some reviewers to one or the

other of the author's interpretations. This would not in the least affect the significance and value of the book, which constitutes a unique presentation of the meaning of individuality on the basis of present knowledge in biology. A wealth of information for the general pathologist and the biologist is provided by this book, which is particularly valuable since the author deals in an unusually objective manner with the results of other investigators and their views. A discussion of the many problems of immunity and genetics dealt with in full and correlated with the author's concept would be beyond the scope of this review. The book will serve as a stimulus to further investigations by open-minded experimentalists. To the general pathologist it is another step in the further development and modification of the concepts of Virchow. The student in the fields of transplantation and tissue and tumor growth will welcome this comprehensive and for the pathologist indispensable work.

**A Textbook of Pathology** By Robert Allan Moore, Edward Mallinckrodt professor of pathology, Washington University School of Medicine, St. Louis. Pp 1338, with 513 illustrations. Price \$10. Philadelphia and London: W. B. Saunders Company, 1944.

To justify its appearance in times like these a new textbook should have great merit. In the opinion of the reviewer this book is more than justified.

The division of the subject matter into general and special pathology, usually found in textbooks of pathology, is followed in this book, but the relative amount of space devoted to each is unusual. General pathology is covered in only 207 pages, while 1,084 are devoted to special pathology. The organization of the material in both sections is new, as is the manner of presentation.

In the part on general pathology the greatest innovation is in the manner of dealing with the retrogressive and degenerative changes. Here the classification is not morphologic but chemical. Chapters are devoted to disturbances in the metabolism of proteins, carbohydrates, lipids and minerals and in the fluids of the body. The organization of the material on inflammation, tumors and disturbances in circulation is more conventional, but the emphasis is on the physiologic and chemical aspects.

The material in special pathology is divided on the basis of whether the cause is known. The diseases of known cause are discussed in parts devoted to those caused, respectively, by living agents, physical agents, chemical agents and deficiencies and those related to pregnancy and the fetal and newborn states. The diseases of unknown or obscure cause are presented in the last part of the book, where they are taken up by systems in the usual way. This is the largest part (544 pages), and together with that on diseases caused by living agents (398 pages) it constitutes most of the volume.

This organization of material has numerous advantages, but it leads to some difficulties and inconsistencies. Thus discussions of diseases of some particular organs are located in widely separated chapters, for example, the renal diseases. Diseases caused by living agents are classified in various ways according to portal of entry, source of the infection, class of organisms or method of transmission. In general this works out well, but some diseases logically belong in several places, having more than one portal of entry, method of spread, etc., and others get misplaced. Thus, although pneumonia is the chief lesion produced by *Klebsiella pneumoniae*, this organism is discussed with the intest-

tinal bacteria. At other times diseases become misplaced because there are not enough chapter headings to go around. Thromboangitis obliterans and Raynaud's disease are placed in the chapter on arteriosclerosis, to the possible confusion of the student.

This book is remarkably comprehensive and up to date. Some of the exotic infections are presented in considerable detail. The style of writing, while not always fluent, is lucid, readable and to the point. The advisability of beginning the presentation of new diseases abruptly with the pathologic anatomy might be questioned on pedagogic grounds. There are numerous references at the end of each chapter and, what is a commendable innovation, the names of pertinent journals.

This book has so many good points and it represents such a laudable effort to inject new life into the subject by a new approach that the reviewer hesitates to point out some minor deficiencies. The author of a textbook must make many decisions on the apportioning of space to the various subjects, and with his decisions others may disagree. Whether 46 pages should be devoted to syphilis and only 28 to tuberculosis and 46 to tumors may be questioned. The infectious granulomas are not presented as a group. The wisdom of abandoning this classification is questioned. In the presentation of edema, the factors in its causation are given, but there is no discussion of the general types, the hazards to the individual patient or the chronic effects in the tissues or serous cavities. In the discussion of hemorrhage there is no mention of the effects and the fate of extravasated blood. The author is impressed by the importance of tularemia pneumonia but not by that due to the Friedlander bacillus.

Some additional minor criticisms could be made. For example, while the illustrations on the whole are excellent, a few could probably be improved (figs 115, 46, 92 and 369b). Figure 350 may well represent metastasis rather than carcinoma. The primary lesion of anthrax is not a pustule, and this disease is contracted also from sheep and goats and their products. Table 4, page 163, is not truly representative of the systemic distribution of neoplasms.

There are numerous additional points on which issue might be taken with statements of fact or on the degree of emphasis. If there is any considerable demand for changes, they will, no doubt, be made by the author in his second edition.

The author of a textbook of pathology is confronted with several important decisions. Shall it be written primarily for medical students or at the reference and postgraduate level? Shall it be the work of one person or of a number of contributors? Shall it be confined to one volume? Dr Moore has apparently attempted to compromise with regard to his audience. The book will probably prove to be more useful to the general student than to the professional pathologist because it often fails to give specific, pathognomonic details for diagnosis, so much desired by the latter. On the other hand, it does present for the pathologist many new points of view. Although the book was written by one author, he acknowledges the help of many consultants and associates. The book is in one volume. It is a heavy book of 1,338 pages. It might well be expanded to two volumes in future editions, with greater emphasis on general pathologic principles.

Here, then, is a new book which should find an important place for itself among textbooks of pathology. The author is to be congratulated, and students of pathology may consider themselves fortunate.

**The Avitaminoses. The Chemical, Clinical and Pathological Aspects of the Vitamin Deficiency Diseases.** By Walter H Eddy, Ph D, emeritus professor of physiological chemistry, Teachers College, Columbia University, and Gilbert Dalldorf, M D, pathologist of the Grasslands and Northern Westchester Hospitals, Westchester County, New York. Third edition. Price \$4.50. Pp 438, with 47 illustrations. Baltimore: The Williams & Wilkins Company, 1944.

The first edition was published in 1937 and was reviewed in that year (*ARCH PATH* **24** 409, 1937). The present edition has nearly 100 more pages and 20 more illustrations.

Part I deals with the chemical nature of the vitamins, vitamin behavior, vitamin requirements, the nature and the function of vitamin A, thiamine, riboflavin, nicotinic acid, pyridoxine, biotin, inositol and vitamins C, D, E and K. It contains many useful tabular summaries of vitamin units, dietary requirements, etc.

Part II presents in detail the etiologic factors, the experimental aspects, the clinical manifestations and the morphologic aspects of the human avitaminoses. In this part are 47 plates of, as a rule, excellent black and white illustrations of gross and microscopic appearances, nearly one half of which appear to be original. Plate 29 needs better description.

Part III describes methods of vitamin assay and tests for vitamin deficiency diseases.

The bibliography of parts I and II is arranged alphabetically according to chapters. The appendix contains a list of references to the estimation of vitamin potencies and two tables of the vitamin values of raw foods. The book contains a vast amount of information about vitamins and their relation to health and disease.

**The Pathology of Internal Diseases.** By William Boyd, M D, LL D, M R C P (Edin), F R C P (Lond), Dipl. Psych, F R C S, professor of pathology and bacteriology in the University of Toronto, Toronto, Canada. Fourth edition, thoroughly revised. Pp 857, with 374 illustrations. Price \$10. Philadelphia: Lea & Febiger, 1944.

The first and second editions of this book were reviewed in earlier volumes of the *ARCHIVES OF PATHOLOGY* (**11** 682, 1931, **20** 965, 1935). There is no change in the general plan and scope of the book. As stated in the review of the second edition, the field covered is that "usually included under the term internal medicine except that acute infectious diseases, such as measles, scarlet fever, smallpox, tularemia, undulant fever and whooping cough, have not been included." It should be noted also that tropical diseases and diseases of the mouth, the pharynx, the esophagus, the vermiform appendix, the peritoneum, the sex organs and the skeleton are not considered. In the new edition several sections, notably those dealing with the cardiovascular system, have been largely rewritten, and much new material has been added. The book is well abreast of recent advances in its spheres of pathology. Of the new topics, alloxan diabetes and Rh factor have not found their way into the index. Four new colored plates and 22 new text figures have been added. The book is well illustrated. In the case of the colored plates the magnification is not given, and no mention is made of the stains in plates 1, 3 and 7. Within its field the book continues to be an excellent guide to the study of the pathology of internal diseases. The statements

on page 744 about the infectiousness of the patient with poliomyelitis and of the carrier of the virus of poliomyelitis are a rare instance of greater finality in the presentation than is warranted by present knowledge

**Lead Poisoning** By Abraham Cantarow, M.D., associate professor of medicine, Jefferson Medical College, and assistant physician and biochemist, Jefferson Hospital, Philadelphia, and Max Trumper, Ph.D. Lieutenant Commander, H-V(S), U.S.N.R. Naval Medical Research Institute, Bethesda, Md. Pp 264 Price \$3 Baltimore The Williams & Wilkins Company, 1944

The absorption, the transportation, the deposition and the excretion of lead form the topic of the first chapter. Then come discussions of the pathology and pathologic physiology of lead poisoning, its clinical manifestations and treatment. The principal part of the chapter on treatment consists of a description by Max R. Mavers, M.D., of the industrial control of lead poisoning. There are chapters also on the occurrence of chronic plumbism, on the normal intake of lead, on lead in body fluids, blood and excretions, on lead products in industry (L. H. Schroeder, National Lead Company) and (by Morris B. Jacobs) on procedures for the determination of lead. There are many tabulations of useful data concerning lead and lead poisoning. There is an extensive bibliography, titles not given, arranged alpha-

betically according to authors' names. The book will be of interest to all medical men who are concerned with the prevention, the recognition and the treatment of lead poisoning.

**Spina Bifida and Cranium Bifidum. Papers Reprinted from the New England Journal of Medicine with the Addition of a Comprehensive Bibliography, from the Department of Surgery of the Children's Hospital, Boston, and Harvard Medical School.** By Franc D. Ingraham, M.D., assistant professor of surgery, Harvard Medical School, neurosurgeon, Children's Hospital, senior associate in neurosurgery, Peter Bent Brigham Hospital. Pp 216 illustrated Cambridge, Mass. Harvard University Press, 1945

The title well describes the contents. The articles discuss the results of observations of the malformations in question in 546 patients seen at the Children's Hospital Boston during the last twenty years. A survey of the cases is presented including the surgical treatment. Three articles deal with occult spinal disorders, an unusual nasopharyngeal encephalocele, and the Arnold-Chiari malformation. The larger part of the book, 128 pages, contains the bibliography from 1556 to 1943 arranged by decades except for the early period. There is here a contribution of great value to those who are interested in the study and understanding of spina bifida and cranium bifidum.

## Books Received

REPORT OF THE NATIONAL ACADEMY OF SCIENCES FISCAL YEAR 1942-1943. Pp 165 Price 25 cents Washington, D. C. United States Government Printing Office, 1944

HOSPITAL FOR JOINT DISEASES NEW YORK THIRTY-SIXTH ANNUAL REPORT—FOR THE YEAR 1943 Pp 102

NATIONAL RESEARCH FELLOWSHIPS, 1919-1944 Administered by the National Research Council. Compiled by Neva E. Reynolds, recording secretary. Pp 142 Washington, D. C. National Research Council, 1944

ARCHIVOS DE LA SOCIEDAD ARGENTINA DE ANATOMIA NORMAL Y PATOLOGICA Dirigidos por D. Brachtetto-

Brian Tomo V. Ano 1943 Pp 143, illustrated Buenos Aires Asociacion Medica Argentina, 1943

REPORT OF THE HENRY PHIPPS INSTITUTE OF THE UNIVERSITY OF PENNSYLVANIA FOR THE PERIOD 1942-1943 Pp 20

PATIENTS HAVE FAMILIES Henry B. Richardson, M.D. associate professor of clinical medicine, Cornell University Medical College, attending physician, New York Hospital, visiting physician, Bellevue Hospital. Price \$3 Pp 408 New York The Commonwealth Fund, 1945

## MORPHOLOGIC ALTERATIONS OF THE NEURON DUE TO TUMOR INVASION

KARL STERN, MD, AND GUY L ODOM, MD

MONTREAL, CANADA

What changes does the nervous parenchyma undergo under the impact of invasion by neoplastic tissue? This question is interesting from several points of view. It is of theoretic interest from the point of view of the pathology of the neuron and from that of the biologic behavior of the tumors which invade nervous tissue. It is of clinical interest because general experience shows that the localizing syndromes pointed out by classic neurology on the basis of vascular lesions or mechanical injuries are often largely modified by tumors occurring in the same area. This discrepancy has been pointed out particularly with respect to the extrapyramidal ganglions in 25 cases from Cushing's department (Ody<sup>1</sup>) and in connection with tumors of the thalamus (Smyth and Stern<sup>2</sup>). Scherer<sup>3</sup> studied the changes which the nervous parenchyma undergoes under the influence of pressure from an adjoining meningioma. In the present investigation an attempt was made to study the effect of glioma on the nerve elements

### MATERIAL AND METHODS

Only postmortem material was studied. It was derived from 15 cases of astrocytoma (piloid, gemistocytic, diffuse), 11 cases of glioblastoma multiforme, 3 cases of astroblastoma, 3 cases of spongioblastoma unipolare, and 4 cases of medulloblastoma—altogether 36 tumors.

The following stains were applied: hematoxylin—Van Gieson (general survey), Nissl (ganglion cells), Mallory's phosphotungstic acid hematoxylin (neuroglia fibers), Bielschowsky (axis cylinders and neurofibrils), Bodian (nerve fibers and nerve endings), Spielmeyer (myelin sheaths), sudan IV (fat), Marchi (degenerated nerve fibers).

### RESULTS

Instead of describing single cases we may summarize the observations as follows:

*Nerve Cells*—The nerve cells are well preserved in an outer zone of the glioma which varies in depth. They become rarer toward the

"interior" part of the neoplasm, but even there one still finds in every type occasional nerve cells which are conspicuously well preserved. In the majority of the cases no transitional stage can be seen between complete preservation and complete disappearance. Where such a change can be seen at all it is of the variety of "simple shrinkage" (Spielmeyer<sup>4</sup>), i. e., a diminution of volume with closer packing of the Nissl bodies and fair preservation of the nucleus (fig. 1). This zone of transition is small in every case and includes only a comparatively small number of cells. The most striking phenomenon is a negative one, i. e., the complete absence of degenerative changes, familiar to all from circulatory, infective, toxic and traumatic conditions, such as the "severe" cell change, the appearance of "homogenization" and the "ischemic" cell change. All these degenerative changes, characterized by various stages and degrees of disintegration of the nucleus, chromatolysis and "liquefaction" are absent.

This is especially striking in certain areas where otherwise the cells show high vulnerability, e. g., the Purkinje cells. These cells, which are known to react even to minor damage with "homogenization" of protoplasm and nucleus, show a completely normal appearance or simple shrinkage when completely engulfed by the tumor cells of medulloblastoma. Even when they are markedly displaced, "carried away" as it were by the growth, they do not show any other changes.

Another instructive example is the nerve cells of the substantia nigra. Because of the melanin, these cells were always particularly valuable for the study of disintegration of ganglion cells in general (Klarfeld<sup>5</sup>, Spatz<sup>6</sup>, Stern<sup>7</sup>). The pig-

4 Spielmeyer, W. Histopathologie des Nervensystems, Berlin, Julius Springer, 1922.

5 Klarfeld, B. Ztschr f d ges Neurol u Psychiat 77 80, 1922.

6 Spatz, H. Encephalitis, in Bumke, O. Handbuch der Geisteskrankheiten, Berlin, Julius Springer, 1930, vol. 11.

7 Stern, K. Ztschr f d ges Neurol u Psychiat 148 753, 1933.

From the Department of Neurology and Neurosurgery, McGill University, and the Montreal Neurological Institute.

1 Ody, F. Arch Neurol & Psychiat 27 249, 1932.

2 Smyth, G. E., and Stern, K. Brain 61 339, 1938.

3 Scherer, H. J. Rev neurol 66 307, 1936.

ment granules enable one to trace parts of the protoplasm throughout all stages of degeneration, neuronophagia and removal to the perivascular space. In a case of piloid astrocytoma of the midbrain, trial sections were taken from various levels. Intact ganglion cells of the substantia nigra were standing out in the depth of the neoplasm, densely encroached on by tumor cells.

forms of necrobiotic change occurring in ordinary brain tissue could be observed.

Stains of intracellular fibrils (Bielschowsky) showed no particular changes.

*Axons and Myelin Sheaths*—Where white matter is involved the myelin stains show the following picture. At the periphery of the tumor the nerve fibers are slightly separated, as it were,

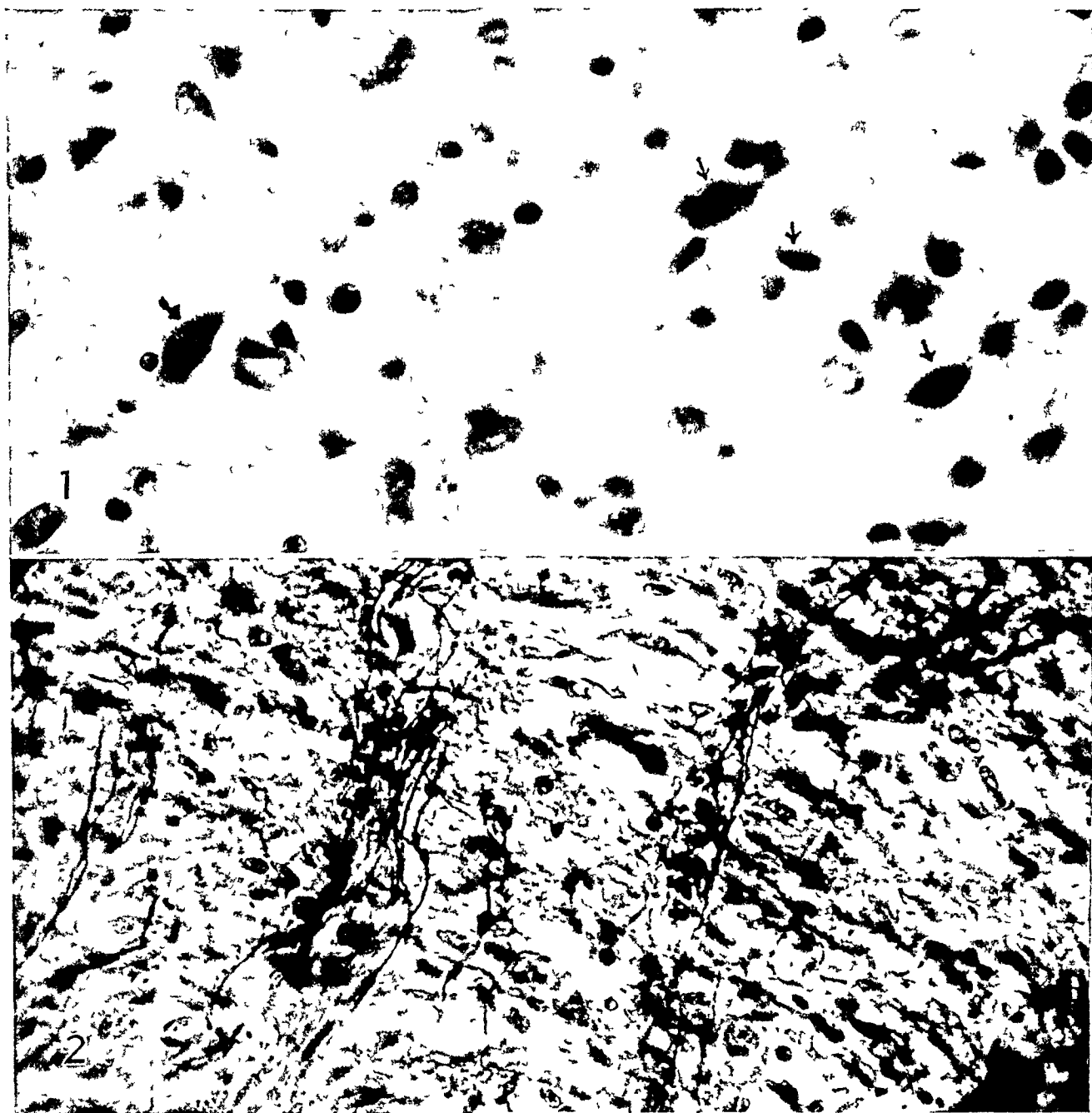


Fig 1—Simple shrinkage of nerve cells within piloid astrocytoma (Nissl stain)

Fig 2—Glioblastoma multiforme growing in white matter (Spilmeyer stain). In the outer zone of the tumor one can see bundles of beaded and tortuous myelinated fibers. They are separated by the growth, but the parallel course can still be recognized.

There were a few clumps of melanin outside ganglion cells, free in the tumor, but not more than one might expect in a normal midbrain of an adult. The common degenerative changes of nerve cells mentioned were seen only in tumor areas which had undergone necrosis. There all

by tumor cells. As one approaches the center of the tumor, there are still bundles of myelin sheaths with maintenance of a more or less parallel arrangement. However, these bundles are separated by masses of tumor cells (fig 2). The myelin sheath itself shows thinning, swelling

and beading at irregular distances. In still deeper zones of dense growth one sees a picture very characteristic of this beading, with fragments of nerve fibers, which are quite irregularly arranged (fig 3). The whole picture resembles dry twigs on the ground. Not all of these are real fragments. Some of them seem to be irregularly bending fibers which have been cut at

unable to obtain a positive Marchi reaction. Some glial cells contain occasional dioplets of neutral fat, but this is obviously not a result of phagocytosis of products of myelin degeneration.

The axis-cylinders show the same changes as far as the irregular arrangement and beading are concerned.

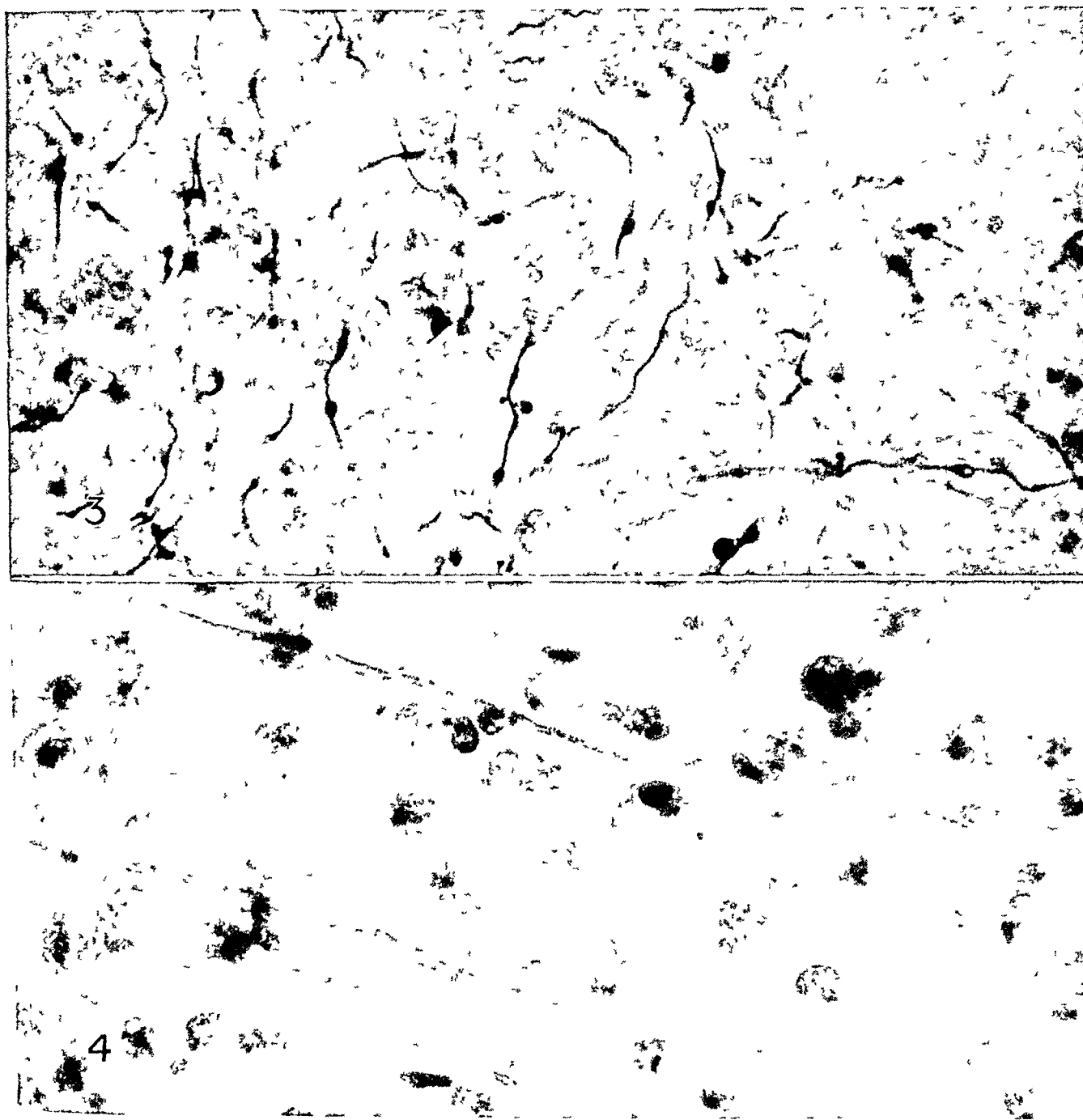


Fig 3—A deeper zone of the tumor seen in figure 2 (Spielmeyer stain). The beaded, thinned, fragmented myelin fibers present the appearance of scattered "dried twigs."

Fig 4—Astrocytoma. The section is from the depth of the tumor. Stained with Sudan IV, the thinned myelin fibers present the normal pale pink appearance, there is no neutral fat reaction.

different levels. However, the most important feature seems to be that even here these severely altered nerve fibers still give a myelin reaction and fail to show any fatty degeneration (fig 4). They stain pale pink except for an occasional bright red droplet with Sudan IV. We were also

All types of glioma showed essentially the same types of change. There is, however, a certain difference in the severity of change which coincides with the malignancy of the tumor. Thus, in glioblastoma multiforme the "outer" zone of well preserved neurons is smaller than in

piloid astrocytoma. The diffuse astrocytoma assumes a peculiar position because of the fact that the nervous parenchyma appears morphologically intact throughout.

#### COMMENT

It has often been emphasized that the various necrobiotic changes of nerve cells described in detail by histologists of the Nissl school can be produced by different vascular, toxic and infective causes, these changes have little specific etiologic significance in themselves. It seems the more remarkable, therefore, that they are never found in nervous tissue invaded by tumor unless necrosis or hemorrhage has occurred in the tumor itself. If any change can be seen at all, it is of the type of "pure atrophy" (Spielmeyer<sup>4</sup>), simple shrinkage of the nerve cells with fair preservation of nuclei and with thinning, beading and fragmentation of axons and myelin sheaths. There are scarcely any signs of degeneration and active removal of neutral fat. In our observations there is nothing to indicate that the presence of occasional droplets of neutral fat in tumor cells is the result of phagocytosis of invaded nervous tissue.

In neuropathology the only processes analogous to this "simple wasting" of neurons are the system degenerations of gray matter (Spielmeyer<sup>4</sup>), such as certain cerebellar atrophies or Huntington's chorea. Moreover, in those conditions, too, the products of degeneration and signs of removal are conspicuously scarce or entirely absent, although it is known that the diseases are progressive.

The only thing which these two types of conditions have in common is the slowness with which the nervous tissue is destroyed. For even the most rapidly growing tumor damages nervous tissue infinitely slowly as compared, for example, with an infarction. In fact, it seems that all the changes described in our cases can be explained on the basis of gradual mechanical compression and distortion of neurons. Only when the tumor itself undergoes necrosis do we see the nerve cell changes commonly associated with anoxia.

This is especially interesting in connection with Scherer's<sup>3</sup> observations on the cortex underlying meningeal tumors. He described various necrotic changes similar to those seen in circulatory diseases, and he interpreted them as due to interference with the blood supply. He contrasted this with the fact that the cortex overlying a glioma does not undergo the same necrotic changes, he argued that since the sup-

plying blood vessels reach the cortex from the pia mater, they can be interfered with only by meningioma. Apart from the fact that this is not quite correct (the cortex is partly supplied by ascending arteries), it seems reasonable that glioma which invades nervous substance in the vicinity of the supplying arteries and emerging veins would cause necrosis in the tissue supplied by these arteries. However, this is not the case. From the present observations one must conclude that a blood supply sufficient to nourish the neoplasm is also sufficient to nourish the invaded nervous tissue and that the destruction of neurons inside the glioma is due to simple pressure atrophy. What happens to entire hemispheres in the case of hydrocephalus happens here to single cells and their processes when these are encroached on by tumor tissue. MacCallum<sup>5</sup> in discussing the contrast between the liver in typhoid and the liver densely infiltrated by cells in myeloid leukemia, drew attention to the fact that no necrosis can be seen in the leukemic liver in spite of the huge infiltration. He stated that in leukemia the liver cells are merely "compressed into flattened rows or squeezed out of existence." This corresponds precisely to our observations on nervous tissue invaded by glioma.

The present investigation explains why glioma seemingly replacing brain tissue can be so exceedingly poor in localizing symptoms and why these symptoms often make their first appearance in an "apoplectic" manner, i. e., after necrosis or hemorrhage in the neoplasm. From a morphologic point of view a surprisingly great number of neurons completely surrounded by neoplasm are still intact. On the other hand the preservation of function can also be explained by the fact that destruction is slow as compared with that in other types of damage. This is especially suggestive in connection with Ody's<sup>1</sup> observation. In his cases tumors of the basal ganglions never caused extrapyramidal symptoms, such as chorea and parkinsonism. It is a common experience that tumors frequently cause pyramidal symptoms. This would suggest that slow invasion of a system of complicated chains of short neurons leaves sufficient time for "detour" conduction of stimuli whereas in the case of one single long neuron no such possibility exists. However this is only offered as a speculative explanation.

The present investigation also indicates that nerve elements deep within the tumor do not

<sup>5</sup> MacCallum, W. G. A Textbook of Pathology, Philadelphia, W. B. Saunders Company, 1939.



in case of doubt justify the assumption of a neuroblastic origin of the neoplasm

#### SUMMARY

In cases of various types of glioma the effect of the growth on invaded nervous parenchyma was studied. All types of glioma produce in this respect essentially the same picture although this may differ in degree. Completely intact neurons can be seen over a varying depth within the tumor. Frequently there is no transition

between complete preservation and complete disappearance of nerve cells. Where there is a change of nerve cells at all it is of the variety of simple atrophy, which is probably due to pressure. There are peculiar changes of axons. All those changes of cells and processes frequently encountered in vascular, toxic and infective conditions are entirely absent unless the neoplasm itself undergoes secondary changes. The foregoing observations have pathologic and clinical significance.

# MORBID ANATOMY OF TYPHUS AS SEEN IN A RECENT GUATEMALAN EPIDEMIC

## OBSERVATIONS ON THE DISEASE

MAJOR ALFRED GOLDEN

MEDICAL CORPS, ARMY OF THE UNITED STATES

On April 3, 1944 epidemic typhus broke out in the Asilo de Alienados (hospital for patients with mental diseases), adjacent to the General Hospital, in Guatemala City. Through the cooperation of Drs. Julio Roberto Herrera and Enrique Padilla of the Sanidad Publica of Guatemala, numerous blocks of autopsy tissue fixed in solution of formaldehyde were forwarded to me for study at the Army Medical Museum, Washington, D. C. A total of 10 deaths were investigated by that method. The public health aspects of this outbreak have been described by Herrera.<sup>1</sup> Martinez Duran<sup>2</sup> has described certain of the pathologic aspects. The purpose of this paper is to draw attention to some interesting features of the morbid anatomy as seen in the tissues submitted, particularly those features which have not received heretofore the attention which I believe they deserve.

### MATERIAL AND METHODS

Certain organs and tissues were not represented in the sample tissues available. For example, no blocks of the endocrine glands other than the adrenal glands were submitted. Only occasional blocks of the gastrointestinal tract were resected for study. On the other hand, multiple blocks of the myocardium, the central nervous system, the liver, the spleen and the kidney were submitted from each case.

It is proposed to present the observations on the available tissues by systems and organs. All sections were stained with hematoxylin and eosin. In 1 case organisms consistent with morphologic descriptions of *Rickettsia* were observed in the testicular tissues after staining the sections overnight in Giemsa solution until the cytoplasm of the swollen endothelial cells lining small blood vessels became deep blue and then decolorizing until the cytoplasm was faintly blue, but retaining the sharp delineation of cell borders. With this method of staining the forms compatible with *Rickettsia* were deep blue and were seen against a nongranular pale blue endothelial cytoplasm.

Suggestions as to technic were contributed by Dr. Arnold Rich, the technical procedures were carried out by Sergeant I. Wodinski.

This study was made possible through cooperative effort of the Institute of Pathology of the Army Medical Museum, Washington, D. C., the Division of Health and Sanitation, Office of the Coordinator of Inter-American Affairs, Washington, D. C., and the Inter-American Cooperative Public Health Service in Guatemala.

1 Herrera, J. R. Bol. Ofic. san. panam. 23: 603, 1944.

2 Martinez Duran, C. Juventud med., 1944, p. 1, Bol. Ofic. san. panam. 23: 791, 1944.

### OBSERVATIONS

*Cardiovascular System*—Of the numerous blocks of myocardium submitted, most of them from the left ventricular wall, many but not all showed variably severe interstitial myocarditis. In 4 cases only one or a few blocks of heart muscle showed the lesion while others did not. On the basis of such distribution it is assumed that interstitial myocarditis was a common if not universal lesion in this epidemic, but patchy in its distribution in a given heart. Most of the muscle fibers in affected zones were rather widely separated by both cellular infiltrations and edema. The infiltrating cells were predominantly mononuclear forms—monocytes and lymphocytes—with lesser numbers of polymorphonuclear leukocytes and only occasional eosinophils. Occasionally a necrotic muscle fiber or a fiber showing necrosis of a portion of its length could be identified, characterized by swelling of the muscle substance, loss of striations and deep homogeneous hematoxylin staining of the ground substance. In contrast with sections of brain and testicle in which blood vessels showing typical reaction to rickettsial parasitization were numerous and seen with particular ease, those of heart muscle showed relatively few vascular lesions. While serial sections were not made in order to explore thoroughly the relationship of vascular lesions to interstitial myocardial inflammation, multiple step or interval sections were examined in 3 cases without finding any evidence that the two were closely related. It appears reasonable to assume therefore that interstitial myocarditis as seen in this epidemic represents a primary insult to the heart muscle and not necessarily an interstitial cellular response to vascular injury.

Lesions of the smaller vessels could be found in all the tissues examined but were particularly plentiful in blocks of the central nervous system, including the brain stem and the spinal cord. Most commonly, but not universally, small arteries and arterioles were the branches of the arterial tree involved. The lesions varied considerably in degree in different cases and in different blocks from the same case. Simple endothelial swelling, varying from slight to

marked, and the formation of two and even three layers of endothelial cells were the most common lesions. Occasionally a fresh hyaline thrombus could be seen attached to the swollen endothelium, partially or completely blocking the lumen (fig 7). The degree of endothelial swelling was estimated as a two to five fold increase of cell volume. The regional cellular reaction to the vascular injuries will be described in connection with the various organs and tissues.

There was no opportunity in this survey to examine portions of major veins, arteries or aorta.

In 1 case extensive fresh thrombosis of the endocardial surface was observed with no evidence of concomitant infarction of the muscle fibers. It was assumed that the thrombus was superimposed on direct endocardial injury.

*Central Nervous System*—In the blocks of the cerebral hemispheres the most common lesion was the vascular response already described. There appeared to be no predilection for any one area or for gray matter as opposed to white matter, except that sections of pons and medulla showed, as a rule, more severe and more numerous lesions than other blocks of the central nervous system. In contrast with the myocardial lesion described, lesions of the central nervous system bore an intimate relation to vascular injuries. While many vascular lesions were surrounded by apparently normal tissue, this was not the rule. Marked vacuolation of nervous tissue regional to involved vessels was common, sometimes extending for as much as the diameter of a low power microscopic field around such vessels. In numerous sections of this series there were several such areas which showed in addition considerable glial mobilization and the presence of compound granular cells. Leukocytes were either scanty or entirely absent in these areas (fig 1).

Around other vessels showing endothelial hyperplasia and hypertrophy there were collars of glial cells ranging from a single row to a few rows of closely packed cells. Other sections showed fresh perivascular hemorrhages, sometimes extending over more than one low power microscopic field and recognizable grossly.

A striking alteration but by no means a universal one were zones of bland necrosis in which the ground substance showed the shadows of the previous living tissue. No cellular reactions were observed around these areas in some instances, and in others mild glial and astrocytic collars were present. The most extensive zone of necrosis was seen in one section of pons (fig 2).

Nodules of Frankel, so frequently noted by many others in epidemic typhus, were found in

profusion in this series. Their relationship to affected blood vessels was frequently obvious. They were of variable composition, ranging from a scattering of glial cells in a zone of partial necrosis to a dense aggregation of a hundred or more such cells with a well defined center of bland necrosis.

The leptomeninges showed round cell meningitis, apparently patchily distributed. In the meshes of the pia-arachnoid, loosened by edema, there were from 5 to 50 round cells per low power microscopic field, chiefly lymphocytes, plasma cells and large mononuclear phagocytes (fig 3).

All of the changes described in the foregoing paragraphs, including zones of necrosis, were seen in sections of the spinal cord in the 2 cases in which the cord was submitted. As in the other portions of the central nervous system, vascular reactions, perivascular zones of necrosis, hemorrhages and nodules of Frankel were distributed apparently haphazardly, affecting both gray and white matter. The meningeal reaction was pronounced in one of these 2 cases and mild in the other.

*Adrenal Glands*—Only 4 pairs of adrenal glands were submitted from this series of cases, and they showed a uniform lesion, varying only in degree in different sections and different cases. The essential features of the lesion were simple necrosis and solution of individual cells or groups of cells, which destroy the continuity of the adrenal gland cords and, when marked, produced lacunas in the substance of the gland. In less extreme examples the normal solid cord became transformed into pseudoglandular foci (figs 4 and 5). Leukocytic reactions were absent in every part of the adrenal gland examined except the periadrenal adipose tissue, where diffuse and focal lymphoid infiltrations were seen with and without specific vasculitis.

The significance of the lesion of the adrenal gland will not be proved until biochemical studies of adrenal gland function can be made in the living patient. It is interesting to note that the same or a very similar lesion has been redescribed recently by Rich<sup>3</sup> in a variety of septicemic states, including meningococcic septicemia. The lesion may be of considerable importance in the genesis of the severe shocklike state so common clinically in Guatemalan typhus.

*Spleen*—The hemopoietic system as a whole cannot be discussed in this paper inasmuch as the only representative of the organs and tissues comprising the system were numerous blocks of spleen. In most of the cases either one or more lesions of the following types could be

<sup>3</sup> Rich, A. R. *Bull. Johns Hopkins Hosp.* 74:1, 1944.

seen (a) hemorrhagic foci or zones and (b) zones of necrosis

In the hemorrhagic areas there were masses of fresh blood covering red and white pulp indiscriminately, without any signs of organization or of undue leukocytic response. These lesions appeared to be terminal phenomena.

The necrotic areas varied from pallid zones a fraction of a low power microscopic field in diameter to those as large as a low power microscopic field. The framework of the pulp, red or white, usually could be recognized, at least in part, as the background for these zones of anemic necrosis.

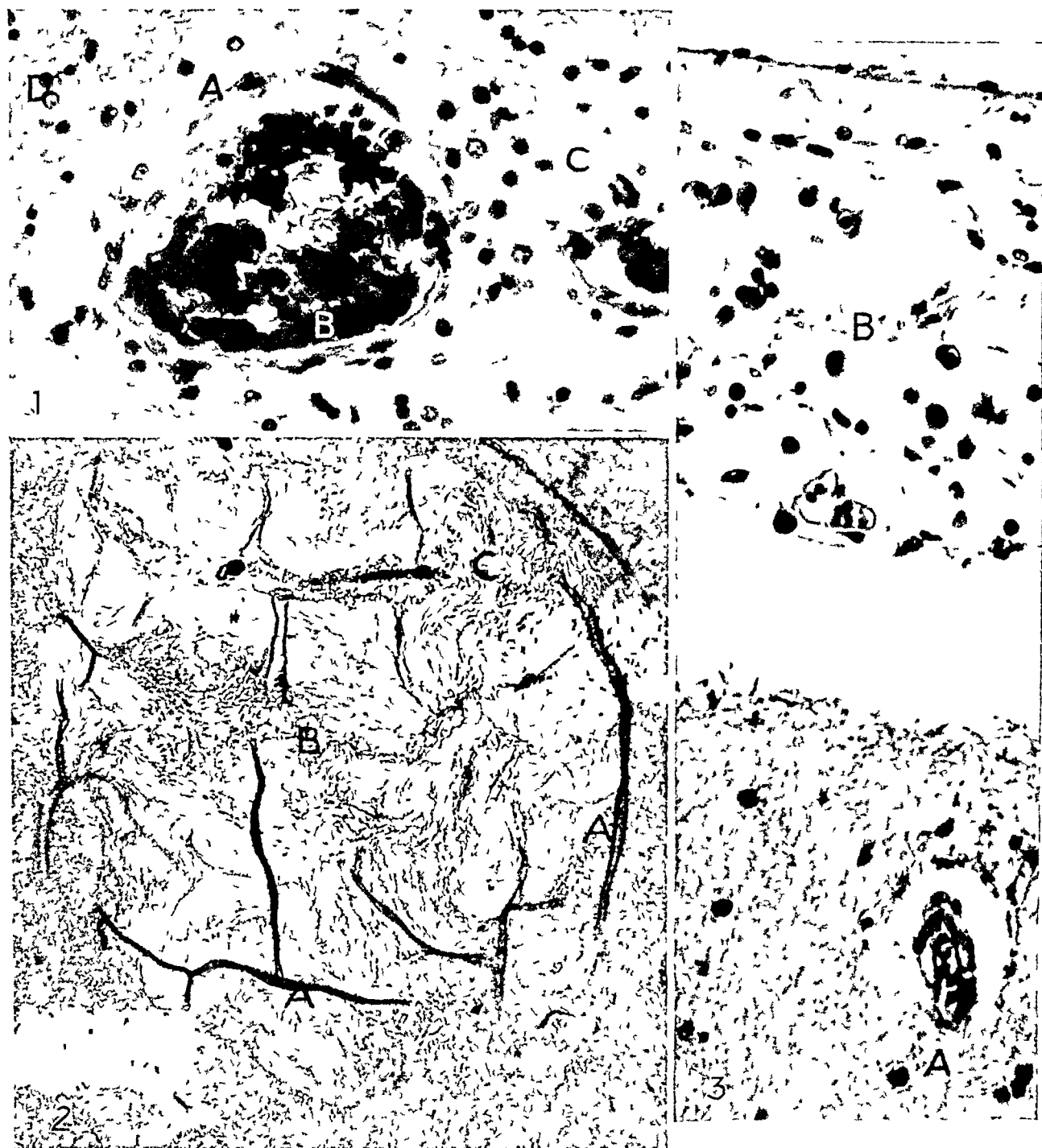


Fig 1—Section of cerebral cortex,  $\times 383$ . Endothelial hypertrophy is seen at *A*. The terminal portion of a thrombus (*B*) partially occludes the lumen. The perivascular tissue shows encephalomalacia (*C*) and contains several compound granular cells (*D*). (Army Medical Museum Negative 80867)

Fig 2—Section of pons,  $\times 43$ . The heavy lines (*A*) are artefacts due to wrinkling of the section. There is a large zone of ischemic necrosis (*B*), along one border of which is a thrombosed vessel with a thick collar of round cells about it (*C*). Note that there is practically no cellular reaction around this large zone of necrosis. (Army Medical Museum Negative 80866)

Fig 3—Section of cerebral cortex showing the pia-arachnoid,  $\times 383$ . A vessel showing marked endothelial hypertrophy is seen at *A*. The pia-arachnoid meshwork is loose and contains numbers of plasma cells, lymphocytes and monocytes (*B*). (Army Medical Museum Negative 80633)

In neither type of lesion could specific vasculitis be incriminated as the cause, although serial or step sections were not undertaken by way of investigation.

In the absence of lesions of either or both of the aforementioned types, the sections of spleen presented no significant changes other than mild and inconstant hyperplasia of pulp cells.

*Liver*—Hypoplasia of cells, chiefly central in distribution, was not uncommon, merging occa-

unlike those seen in typhoid were present, occasionally in considerable numbers. Specific vascular lesions were observed only occasionally and appeared to be confined to the portal spaces, where small granulomatous foci surrounded such vessels, appearing not unlike the nodules of Frankel in the brain.

*Skin*—The superficial portions of the corium of exanthematic plaques showed numerous specific vascular lesions similar in all respects to

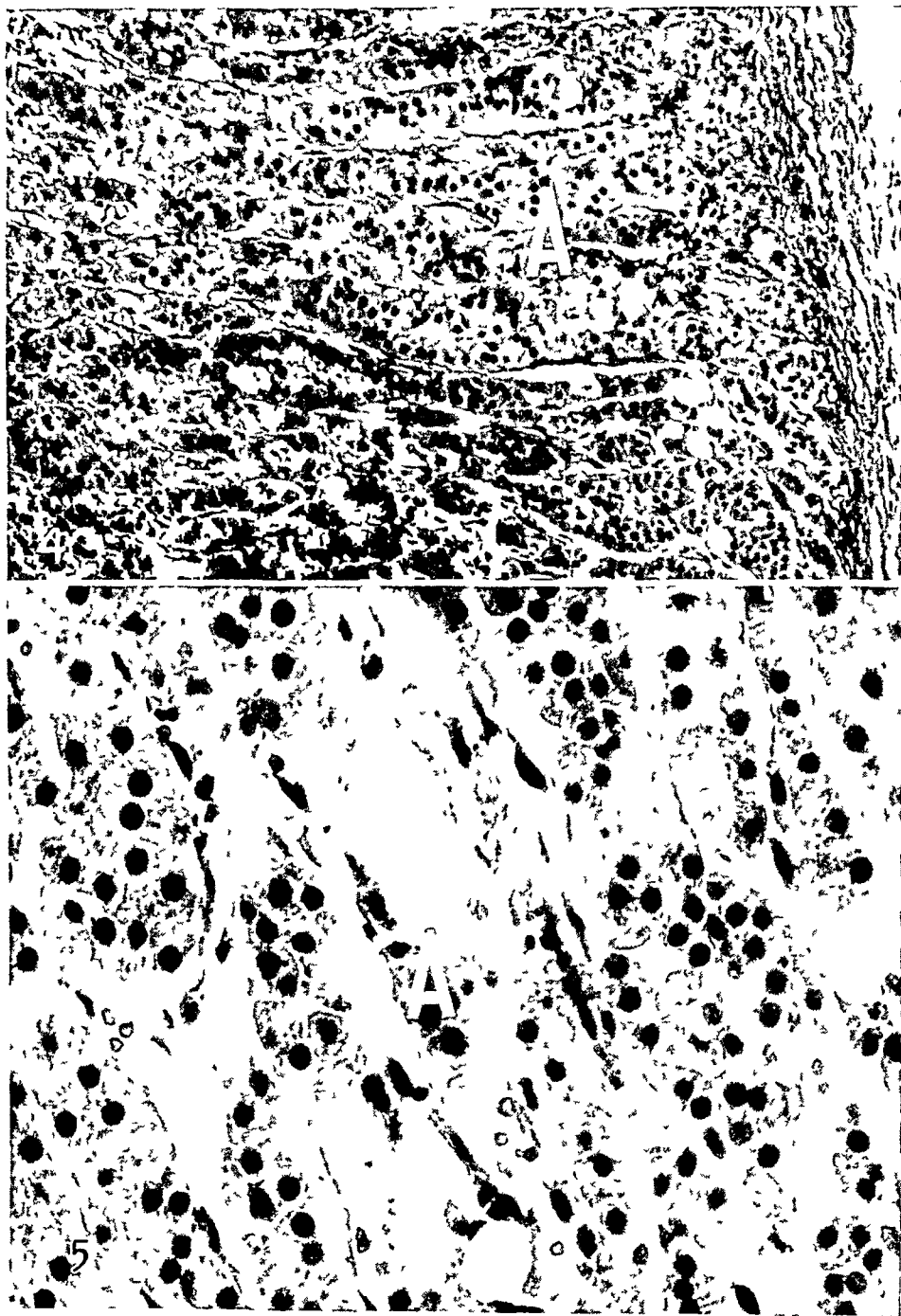


Fig 4—Section of the cortex of an adrenal gland,  $\times 120$ . Necrosis of individual cells has resulted in irregular lacunas (A) dispersed haphazardly in the cortical substance of the gland (Army Medical Museum Negative 80830).

Fig 5—View of a portion of the section shown in figure 4 under higher power,  $\times 400$ . Note the sudden break in the continuity of the adrenal gland cord (A) with solution of a number of cells. Note that the sinusoidal border can be identified in the necrotic area and that remnants of cytoplasm are still visible (Army Medical Museum Negative 80831).

sionally with zones of frank necrosis, without hemorrhage. In other areas focal necroses not

those seen in the brain. Unaffected vessels were usually congested. Lymphangiectasia was com-

mon, even in the vessels lying deep in the corium. Unorganized hemorrhages were seen frequently and tended to be located superficially in the corium. No changes could be seen in the overlying epidermis.

Of special interest was one section presumably from a large macular, perhaps confluent exan-

such that one could predict sloughing of that area in time. It is suggested that a lesion of that type may be of significance in the genesis of typhus ulcers.

*Kidneys*—Numerous blocks showed one or more examples of the lesions to be described, among which specific vasculitis was inconspic-

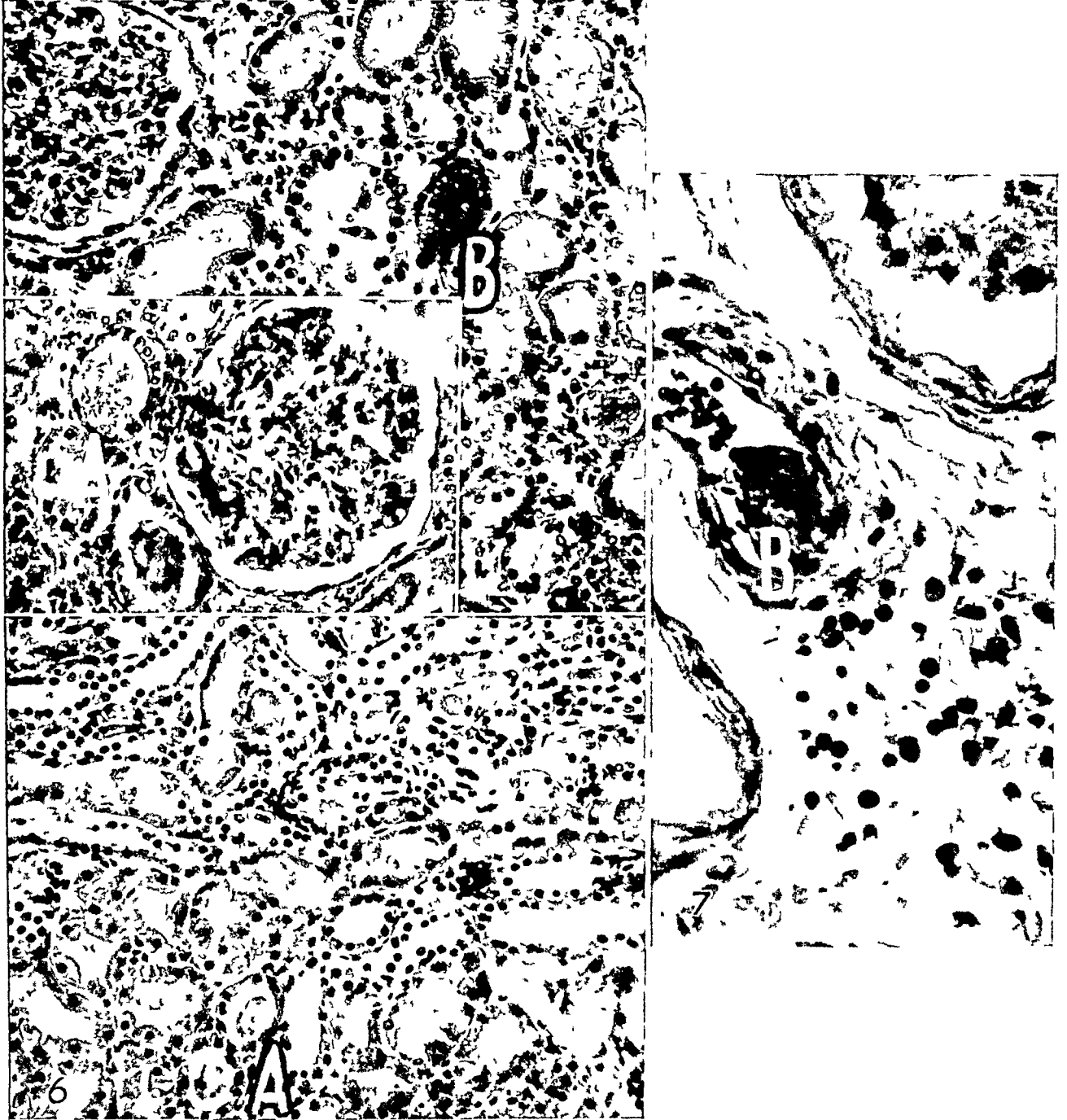


Fig 6—Composite photograph of renal lesions in 3 different cases, showing brown-pigmented casts (A), red blood cell agglomerations (B) and interstitial round cell infiltrations (C),  $\times 220.5$  (Army Medical Museum Negatives 80925, 80927 and 80926)

Fig 7—Section of testicle showing an interstitial vessel with endothelial hyperplasia (A) and thrombus formation (B) with regional round cell infiltration (C),  $\times 383$  (Army Medical Museum Negative 80636)

thematous plaque. The lesion was characterized not only by the specific vasculitis just described but by widespread hemorrhagic necrosis including the epidermis and extending down to the subcutaneous adipose tissue. The lesion was

usual. Yellow to brown homogeneous casts were seen, particularly in the distal portions of the convoluted tubules, and occasionally were present in profusion. In the same sections, as well as in those from different cases, other

tubules had frank agglomerations of red blood cells in similar situations. The pigmented casts were consistent in tinctorial properties with those of hemic origin. Inasmuch as pigmented cast-red blood cell mixtures were also seen, the supposition appears warranted that the lesion represents a hemic or hemoglobinuric nephrosis of undetermined genesis. Simple vascular lesions of the glomerular tuft caused by rickettsial injury were not seen, although occasional hemorrhages in Bowman's capsule were noted. These appeared to be all too slight in degree to account entirely for the tubular lesions. Occasional foci of necrosis of the tubular epithelium adjacent to the casts were present and tended to form minute pseudogranulomatous foci composed of round cells and epithelial cells, spilling over into the interstitial tissues. In addition to the tubular lesions, and in some cases apparently independent of them, there was mild to severe subacute interstitial nephritis characterized by infiltrations of lymphocytes, plasma cells, lesser numbers of monocytes and polymorphonuclear leukocytes, limited neither to the cortex nor to the medulla. There was only minimal and at times no leukocytic infiltration around the pelves or calices, distinguishing the lesion from ascending pyelonephritis (fig 6).

*Testes*—In the 6 cases in which testicular tissues were submitted, all sections showed the specific vasculitis characteristic of rickettsial infection either in the tunica albuginea or in the interstitial tissues of the testicle, or in both areas. If one may judge the extent or the severity of the vascular reaction from this form of random sampling of tissue specimens, it appears that the lesion was particularly severe in the testicle and its tunic. The vascular reaction with partial thrombosis of the vessel and regional interstitial round cell infiltration illustrated in figure 7 is typical of this series. Specific tubular lesions and damage to the interstitial tissues were not observed.

*Esophagus*—Several blocks from the gastrointestinal tracts of 2 patients were the only ones of this organ submitted for examination. Each showed submucosal round cell infiltration, usually in discrete foci and of moderate degree. No ulceration of the mucosa was noted. The significance of the lesion, which again appeared to be independent of specific vasculitis, is obscure.

#### COMMENT

The lesions of louse-borne epidemic typhus as seen in 10 fatal cases in this outbreak may be classified in two large groups. One group consists of the specific vascular lesions of rickettsial infection, which needs no further description. The vascular lesions appeared to be most pro-

nounced in the central nervous system and the testes, and they were responsible apparently for a certain degree of destruction or at least inflammation of the regional tissues. The other group of lesions appeared to be independent of, or at least not intimately related to, the vascular lesions and included interstitial myocarditis, meningitis, focal cellular necrosis of the adrenal glands, interstitial nephritis, hemoglobinuric nephrosis and focal necroses of the liver and spleen. Of particular significance were the observations in the spinal cord, which showed lesions identical with those of the cerebral hemispheres, the cerebellum and the brain stem. The lesions of the pons and the medulla were the most severe, both in number and in extent, and included the large areas of bland necrosis seen in figure 2. It was suggested that the severe degree of hemorrhagic necrosis seen in one section of skin could be the pathogenetic precursor of a typhus ulcer.

The clinical state of collapse so remarkable in typhus in Guatemala may be correlated with focal necrosis of the adrenal glands, although one must also consider interstitial myocarditis and the lesions of the central nervous system in the pathogenesis of that state. One can only speculate on the residua or sequelae of such massive necrosis of the central nervous system as was seen in this series in patients that survive the primary illness. Obviously such necrotic areas could heal only by neural scar formation, with clinical sequelae in direct proportion to the exact site and extent of the part of the central nervous system affected.

Hemoglobinuric nephrosis is of some significance because lesions similar to that which characterize this condition are seen in association with severe body burns, massive crushing of muscles ("crush syndrome")<sup>4</sup> and certain shocklike states. I shall not attempt with the inadequate data at hand to speculate on the exact mechanism of this lesion in typhus. The fact nevertheless remains that it exists to a degree comparable to that seen in body burns, for example. Interstitial nephritis of infectious origin is too well known to require further comment. Apparently, epidemic typhus may be added to the list of diseases in which the lesion may be encountered.

#### SUMMARY

In addition to vascular and other lesions characteristic of rickettsial infection, this study of 10 cases of typhus revealed foci of necrosis in the central nervous system and in the adrenal glands. In the adrenal glands the necrosis was associated with cytolysis.

<sup>4</sup> Bywaters, E. G. L., and Dible, J. H. *J. Path. & Bact.* 55: 7, 1943.



# GENETIC ANALYSIS OF THE INDUCTION OF TUMORS BY METHYLCHOLANTHRENE

## VIII TWO MUTATIONS ARISING IN MICE FOLLOWING INJECTION OF METHYLCHOLANTHRENE

L C STRONG, PH D

NEW HAVEN, CONN

Six genes for susceptibility to methylcholanthrene induction of tumors have been indicated by data which I have recorded<sup>1</sup> These are, in order of discovery, one on the "agouti" chromosome, one on the "pink eye" chromosome, one on the "piebald" chromosome and three on the X chromosome The gene on the "pink eye" chromosome and one of the genes on the X chromosome have been demonstrated in tests for linkage following suitable outcrosses The other four genes are indicated, but the tests for linkage have not been completed The three genes on the X chromosome probably are allelomorphs The interpretation that seems to fit the data best for the three sex-linked genes is that a single gene which did not originally give a greater susceptibility to methylcholanthrene induction of tumors in one or the other sex has mutated twice at some time in the past—once in the direction that produces greater susceptibility in the female and again in the opposite direction, producing greater susceptibility in the male As far as known, these six genes were involved in susceptibility to induction of tumors before the various inbred strains of mice were either established or given injections of methylcholanthrene

During the past five years an experiment has been performed for the purpose of analyzing the genetic factors obviously involved in the induction of tumors by methylcholanthrene For this purpose 200 mice (100 females and 100 males) of each of the fifteen inbred strains<sup>2</sup> were used In addition to these inbred mice several special strains were established through selection following outcrosses Some of the data have been published<sup>3</sup>

From the Department of Anatomy, Yale University School of Medicine

This experiment was made possible by grants from the Anna Fuller Fund and the Jane Coffin Childs Memorial Fund for Medical Research

1 Strong, L C Rec Genet Soc Am **12** 56, 1943, unpublished data (records on three stocks C, I and F not complete)

2 Strong, L C Cancer Research **2** 531, 1942

An analysis of the pertinent data obtained so far discloses that two new germinal mutations have arisen during the course of the experiment in which methylcholanthrene has been injected into hybrid mice It is the purpose of this paper to present the data that indicate the origin and the transmission of these two mutations

### MATERIALS AND METHODS

All mice used in this experiment were of the NHO descent and belonged to the same series as those described in previous reports<sup>3</sup> The NHO mice were originally produced by tandem crosses between mice of the CBA, the JK and the N inbred strains The idea behind the production of the NHO strain was to obtain a group of mice which would show the greatest possible degree of biologic variability without the interfering incidence of spontaneous tumors Up to the present time spontaneous tumors have been infrequent, as reported previously The incidence of all types of spontaneous tumors with the exception of those of the lung is less than 1 per cent Bronchiogenic carcinoma is the only spontaneous tumor to which the NH (or the derived NHO) stock shows any significant susceptibility (starting at 325 days of life and increasing in numbers up to 30 per cent of all mice by 575 days and to 42 per cent at 675 days of life) In a very small number of these mice mammary tumors, adenocarcinoma of the stomach (in one subline), melanotic tumors, leiomyosarcoma of the uterus and leukemia have been observed Cystic ovaries and cystic glandular hypertrophy of the uterus are evidences of hormonal dysfunction in mice of this strain—two conditions which increase in frequency with advancing age Stenosis of the esophagus<sup>4</sup> and glomerulonephritis<sup>5</sup> are of common occurrence in these mice, also

At 60 days (plus or minus 1 day) all mice were given on the right side of the body a subcutaneous injection of 1 mg of methylcholanthrene dissolved in 0.1 cc of sesame oil Mice were kept either as breeders or as

3 Strong, L C Am J Cancer **39** 347, 1940, Cancer Research **1** 572, 1941 Strong, L C, and Williams, W L *ibid* **1** 886, 1941 Strong, L C, Collins, V J, and Durand, E A *ibid* **3** 21, 1943 Strong, L C Arch Path **36** 58, 1943 Williams, W L, and Strong, L C Cancer Research **4** 11, 1944 Strong, L C Arch Path **37** 131, 1944

4 Strong, L C, and Smith, G M Yale J Biol & Med **13** 489, 1941

5 Kirschbaum, A Proc Soc Exper Biol & Med **55** 280, 1944

nonbreeders on a diet of Nurishmix<sup>5a</sup> supplemented with a mixture of wheat and oats, enriched Bond bread<sup>5b</sup> soaked in milk and at intervals washed lettuce. Weekly observations were made on all animals, and as soon as it was apparent that the local tumor was definitely growing or as soon as any disorder was shown, such as emaciation, ruffled hair and wheezing, the involved mouse was killed. Within a few hours after death an autopsy was made on the mouse.

The present survey of tumors occurring in NH or NHO mice after the subcutaneous injection of methylcholanthrene is based on observations of 4,000 mice. The types of tumors observed included, among others, (1) fibrosarcoma, (2) rhabdomyosarcoma, (3) epidermoid carcinoma, (4) adenocarcinoma of the mammary gland, (5) adenocarcinoma of the pyloric end of the stomach, (6) adenocarcinoma of the lungs and (7) carcinoma of the liver. The study of the 4,000 mice (between the  $F_4$  and the  $F_{12}$  generation) was divided into two parts. The first part consisted of observations on 2,000 unselected mice (the NH) treated with methylcholanthrene, that is, every mouse of the early hybrid generations ( $F_4$  to  $F_8$ ) received an injection of the carcinogen. The second part consisted of observations on mice which were selected toward resistance to the induction of tumor at the site of the injection (the NHO mice, 2,000).

In testing for linkage (partially indicated by data obtained in the observations just mentioned) the hybrid mice of the NHO strain from the  $F_{12}$  to  $F_{16}$  generations have been divided into five sublines depending on the coat colors they carry. These are (a) brown nonagouti showing a high incidence of carcinoma of the stomach (both spontaneous and induced by methylcholanthrene), (b) brown nonagouti not showing carcinoma of the stomach, (c) pink eye brown nonagouti, (d) piebald brown nonagouti and (e) pink eye, piebald brown nonagouti.

#### RESULTS

The gene on the "piebald"-tagged chromosome reduces susceptibility to the effects of methylcholanthrene by 10 to 15 per cent. (See chart 1. The rate of induction of tumors in self-colored mice [a] is represented by the short dash curve, the rate in piebald mice [b], by the solid line curve. The rate in  $F_1$  [self-colored mice obtained by a cross between piebald and self-colored mice] [d] is shown in the open circle long dash line. The rate in self-colored mice [f] obtained in the backcross to the recessive piebald stock is given in the solid dot, solid line curve and that in piebald mice [e], in the solid dot, long dash line.) In one descent of the brown nonagouti piebald subline this 10 to 15

per cent reduction of susceptibility no longer occurs. (In chart 1 the rate of induction of tumors in the "mutated" subline [c] is shown in the long dash line.) The stock is homozygous for "piebald." Consequently the phenomenon of "crossing over" cannot explain this loss of resistance to the local induction of tumors by methylcholanthrene. Further tests for linkage in the backcross to the recessive piebald subline have demonstrated that the new mutation has arisen, presumably at the site of the old gene (for local susceptibility to induction of tumors) on the "piebald"-tagged chromosome. That is there is now no differential susceptibility between self-colored and piebald mice.

The second mutation has occurred on the X chromosome. The evidence is as follows. The analysis of the first 2,000 mice of the NHO descent shows that there is no sex difference in susceptibility to the local induction of tumors by methylcholanthrene. (See chart 2. The rate in the original females [a] is represented in the

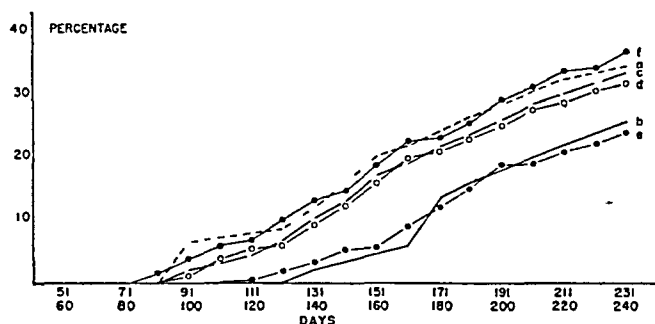


Chart 1—Curves for the rates at which local tumors were induced when 1 mg of methylcholanthrene was injected subcutaneously into (a) a homozygous Brown nonagouti self-colored subline of the NHO descent (short dash line), (b) a homozygous Brown nonagouti piebald subline (solid line), (c) the  $F_1$  generation from a cross between "a" and "b" (long dash line), and (d and e) the two classes of self-colored and piebald mice obtained in the back cross to the recessive piebald subline (self-colored circle, long dash line, and piebald, solid dot, long dash line). Time in days is plotted along the base line, the percentage incidence of local tumors, on the vertical line (cumulative data). The curves indicate occurrence of a linkage between a gene of susceptibility to local induction of cancers and the piebald chromosome. Following this mutation (f) the derived piebald mice have the same susceptibility as the original self-colored mice (solid dot, solid line curve).

short dash line, that in males [b], by the solid line.) In one of the sublines there has appeared a sex difference. (In chart 2 the rate in the females of the "mutated" subline [c] is shown in a solid dot, solid line curve, that in the males of the "mutated" subline [d], in a solid dot, long dash curve.) In this subline the females are more susceptible to the local induction of tumors by methylcholanthrene than the males—a situa-

<sup>5a</sup> Nurishmix, according to the manufacturer, contains crude protein, not less than 20 per cent, crude fat, not less than 6.50 per cent, crude fiber, not more than 3.5 per cent, carbohydrates, 51.50 per cent, nitrogen-free extract, not less than 48 per cent. The ingredients are dried buttermilk, beef scrap, wheat germ meal, rolled oats, calcium pantothenate, molasses, iodized salt (1 per cent) and cod liver oil.

<sup>5b</sup> Bond bread is stated by the manufacturer to contain thiamine, 55 per cent of the minimum daily requirement of man, riboflavin, 17.5 per cent, nicotinic acid, 5 mg, and iron, 40 per cent of the daily human requirement.

tion that is also found in the CBAN stock Tests for linkage in suitable genetic crosses have also disclosed the presence of this altered gene on the X chromosome

That is, when mice of the original NHO strain (2,000 mice) were outcrossed to the C<sub>57</sub> black strain, there were obtained no sexual differences in susceptibility in the F<sub>1</sub> generation This can be explained on the assumption that the genetic constitution of the old NHO strain as far as the X and Y chromosomes are concerned is the same as that of the C<sub>57</sub> strain—X<sup>A1</sup> X<sup>A1</sup> female NHO, X<sup>A1</sup> Y male NHO and X<sup>A1</sup> X<sup>A1</sup> female C<sub>57</sub>, X<sup>A1</sup> Y male C<sub>57</sub>—when the A<sup>1</sup> gene on the X chromosome has no effect on susceptibility On the other hand, when mice of the altered or “mutated” NHO strain were outcrossed to mice of the C<sub>57</sub> strain there was a differential susceptibility between the sexes depending on the way in which the outcross had been made When a female “mutated” NHO mouse was outcrossed to a C<sub>57</sub> male, the

“A” gene acts as a stimulator of local induction of tumors whereas the “A<sup>1</sup>” gene has no effect In a third state the “a” gene acts as an inhibitor of susceptibility<sup>6</sup>

Classification of Data on Which the Curves in Charts 1 and 2 Were Computed

	Curve	Class	Mice
Chart 1	-----	(a) Original self colored	2,246
	————	(b) Original piebald	445
	— — — —	(c) F <sub>1</sub> self colored × piebald	155
	○ — — ○	(d) Self colored BC to b	268
	● — — ●	(e) Piebald BC to b	219
	● — — ●	(f) “Mutated” piebald	261
			Total 3,637
Chart 2	-----	(a) Original females	483
	————	(b) Original males	507
	● — — ●	(c) Females of “mutated” sub strain	194
	● — — ●	(d) Males of “mutated” substrain	179
			Total 1,375
			Grand total 5,012

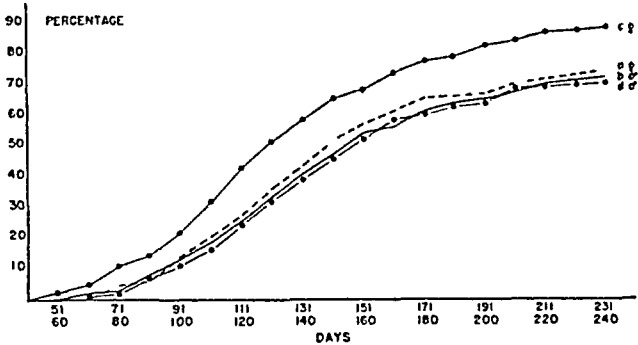


Chart 2—Curves indicating a mutation occurring on the X chromosome, thus changing susceptibility to methylcholanthrene induction of tumors in the female but not in the male Before the mutation arose there was no sex difference in susceptibility—(a) females, short dash line, (b) males, solid line—whereas after the mutation the females became more susceptible to local induction of tumors—(c) females, solid dot, solid line curve, (d) males, solid dot, long dash line curve

F<sub>1</sub> males showed a higher susceptibility than did the females, whereas, when a male “mutated” NHO mouse was outcrossed to a C<sub>57</sub> female, the reverse was true That is, the females now showed a higher susceptibility These facts can be explained on the assumption that the genetic constitution of the “mutated” NHO strain is now X<sup>A</sup>X<sup>A</sup> female, X<sup>A</sup>Y male with A acting as a stimulator of local induction of tumors The genetic constitutions of the F<sub>1</sub> mice would then be X<sup>A</sup>X<sup>A1</sup> female, X<sup>A</sup>Y male in the cross between a “mutated” female NHO (X<sup>A</sup>X<sup>A</sup>) and a C<sub>57</sub> male (X<sup>A</sup>Y) and X<sup>A1</sup> X<sup>A</sup> female, X<sup>A1</sup> Y male when the C<sub>57</sub> female X<sup>A1</sup> X<sup>A1</sup> was outcrossed to a “mutated” NHO male X<sup>A</sup>Y The

COMMENT

The sex-linked gene indicated by data from studies of the inheritance of susceptibility to methylcholanthrene induction of tumors is no doubt an “unstable” one, having apparently mutated at least twice before the establishment of the fifteen inbred strains developed by me and once since the introduction of methylcholanthrene On the assumption that in one condition (A) the gene acts as a stimulator, in another (A<sup>1</sup>) it has no effect on susceptibility and in still another condition (a) it behaves as an inhibitor of the production of tumors, the genetic constitution of the mice of the various inbred strains are X<sup>A</sup>X<sup>A</sup> female, X<sup>A</sup>Y male in the CBAN stock, X<sup>A</sup>X<sup>a</sup> female, X<sup>a</sup>Y male in the CHI and C stocks, and X<sup>A1</sup> X<sup>A1</sup> female X<sup>A1</sup>Y male in the N, A, C<sub>57</sub>, JKL, L, C<sub>12</sub>I, FC, JK CBA and C<sub>3</sub>H stocks The present data indicate that the same gene has again mutated from A<sup>1</sup> to A following the injection of methylcholanthrene The constitution of the new subline would then be X<sup>A</sup>X<sup>A</sup> female and X<sup>A</sup>Y male, the same as in the CBAN strain

We are confronted with the problem of whether the two mutations were spontaneous or were induced by methylcholanthrene The situation cannot be positively demonstrated However, the impression is gained that the presence of methylcholanthrene within the body did have something

6 Strong, L C Unpublished data

to do with the appearance of two mutations within the relatively short period of six months. This impression is based on the following reasons. Germinal mutations in my laboratory have been extremely rare. In twenty-five years 210,000 mice have been raised and personally examined. During this period only eight germinal mutations have been detected. These were (a) albinism in the JK stock, (b) a recessive lethal "piebald" appearance in the CHI stock, (c) a "pink eye" in the C<sub>3</sub>H stock, (d) absence of the xiphoid process of the sternum in the JK stock, (e) an extra claw on the front feet of JK mice, (f) polydactylism in 1 case in which inheritance was uncertain, (g) lop ear in the CHI—inheritance irregular—and (h) waltzing in the C<sub>57</sub> stock. So two mutations within six months seems to be more than should be expected from chance alone, especially when they were in the direction of increased susceptibility to tumor—a physiologic state already known to be influenced by methylcholanthrene somatically. The fact that no other germinal mutations have been reported by other investigators with thousands of animals receiving methylcholanthrene does not seem to invalidate the present interpretation when one takes into consideration that nearly all the work on the induction of tumors by methylcholanthrene in other laboratories has been done on animals and that pedigreed descendants have not been produced and kept (at least not reported). In fact most of the reported work was done specifically on mice not being used as breeders. Again the interest in using mice of the established inbred strains is rapidly gaining impetus. Now most germinal mutations are recessive, and one should not be able to detect them for several generations. My co-workers and I have deliberately used pedigreed hybrid mice and subjected their descendants for several generations to the same treatment of subcutaneous injection of methylcholanthrene. Under these conditions we have been able to detect two germinal mutations that influence susceptibility to the local induction of tumors. The fact that one of the genes which mutated, the one on the X chromosome, is apparently an "unstable" one should not weigh too much against the idea that the change (mutation) may have been enhanced by the presence of methylcholanthrene.

Mottram<sup>7</sup> briefly discussed the idea of carcinogens acting on genes (possibly during mitosis) rather than having a direct effect on living cells. He presented data on the appearance of chromo-

somal aberrations being produced in bean seedlings by carcinogenic tar. Unfortunately he did not distinguish between somatic and germinal mutations. I am not in agreement with Mottram's statement that "the mutation hypothesis for cancer postulates that the cancer cell differs from the normal cell in its content of genes."

In 1926 Strong<sup>8</sup> wrote

changes within the genetic constitution of living forms, if they are germ cells, are called mutations, if they are somatic cells (occurring especially in plant tissues) they are called somatic mutations.

The conclusion is therefore reached that somatic mutations may occur within the tumor cell, and that when these mutations do occur they change the reaction potential and other physiological activities of the tumor cell. The data at hand are not sufficient to determine more in detail the nature of this mutational process. It may be either a change or shifting of a complete chromosome or chromosomes, or a change or changes within a chromosome or chromosomes (genic or it may be even cytoplasmic in nature). By mutation I merely mean to use the term in its broadest sense, that is, a change or shift within the genetic or internal constitution that results in definitely clear cut or discernible differences in behavior or structure that is perpetuated by the process of heredity (in this case—cell division).

There are no data available that would necessitate the changing of this concept in any way. Certainly the new contributions on the mitochondria (Claude), the viruses (Rous, Duran-Reynals and others), extrachromosomal inheritance (Little and others) (Korteweg) and the influence of the milk (Bittner, Andervont) would indicate that a genic change in the conversion of a somatic cell into a cancerous one may be merely the final step and may not necessarily be the only one involved.

The cytologic or genetic changes involved in the origin of cancer under spontaneous as well as under induced conditions are shrouded in mystery. The present data on the appearance of two germinal mutations which underlie cancerous susceptibility are clearcut. The concept may be considered that the role of methylcholanthrene (and perhaps other carcinogens) in the induction of tumors has a twofold nature: (1) It has a direct effect on somatic cells and by some unknown process influences them to such an extent that they become cancerous (somatic mutations?), and (2) it also influences germinal mutations in a manner such that the constitutional states of susceptibility and resistance to cancer are altered and these changed states are transmitted to succeeding generations. The possible relationship of the somatic to the germinal changes in cancer is beginning to be indicated. In one type of neoplasm especially, adenocarci-

<sup>7</sup> Mottram, J. C. Brit J Exper Path **15** 71, 1934

<sup>8</sup> Strong, L. C. Genetics **11** 294, 1926, J Exper Med **43** 713, 1926

noma of the stomach of the mouse," this relationship seems to be a close one. That is, it has been demonstrated that the descendants of mice in which adenocarcinoma of the stomach has been induced by methylcholanthrene now continue to show development of the same condition spontaneously. Perhaps in other cancerous conditions, also, the role of the carcinogen may be of a dual nature—an effect on somatic cells and another somehow or other correlated change in the germ plasma. Either change may take place without the other one doing so.

#### SUMMARY

Two new germinal mutations which change susceptibility to methylcholanthrene induction of tumors have appeared in mice.

One of these mutations appeared on the "piebald"-tagged chromosome, the other, on the X chromosome.

The gene for susceptibility to induction of cancers on the X chromosome is apparently an "unstable" one, having mutated at least twice in the past and once following the subcutaneous injection of methylcholanthrene.

As to the role of methylcholanthrene in the induction of neoplasms, perhaps methylcholanthrene acts in a dual manner: (1) directly on the somatic tissues, possibly bringing about somatic mutations, and (2) on the germinal mechanism which determines in part the intrinsic constitutional states of susceptibility and resistance to induction of cancers. The somatic and the germinal change may be, to some extent at least, correlated and influenced by the same extrinsic stimulus—in this case, methylcholanthrene.

# CHANGES IN THE UTERUS AFTER ERADICATION OF ENDOMETRIAL ADENOCARCINOMA BY RADIOTHERAPY

WITH PARTICULAR REFERENCE TO AN INFARCT-LIKE RADIONECROTIC PLAQUE IN THE LINING

JOHN F. SHEEHAN, M.D., HERBERT E. SCHMITZ, M.D.

AND

JANET TOWNE, M.D.

CHICAGO

We<sup>1</sup> recently published a clinical report of the effects of combined roentgen and radium therapy on adenocarcinoma of the endometrium. In some of the cases hysterectomy was performed at varying intervals of time after the completion of the radiation treatments. Routine microscopic examination of grossly abnormal areas of the uterus in 5 cases revealed no evidence of carcinoma. In 4 of these cases (1 to 4) the uterus was later subjected to a much more thorough examination. There was still no evidence of carcinoma. However, various interesting changes induced by radiation were present, notably an infarct-like plaque of necrosis at the level of the internal os. It is the purpose of this paper to give a detailed description of the changes and to discuss the composition and the genesis of the plaque at the internal os. The observations in 2 other cases (5 and 6), which throw some light on these points, will be summarized also even though only routine sections were available for study.

## REPORT OF CASES

In the previous report<sup>1</sup> items of clinical importance were stressed. In the present paper only the most pertinent clinical data and the doses and the technics utilized in the radiation therapy will be furnished. Additional information can be obtained from the previous paper.<sup>1</sup> In tables 1 and 2 of that communication all 6 of the cases to be reported here are listed. The initials of the patients are used in the earlier report instead of the case numbers, as follows: R. B., case 1, P. G., case 2, D. C., case 3, M. S., case 4, A. D., case 5, A. M., case 6.

From the Department of Pathology and the Department of Obstetrics and Gynecology of Loyola University School of Medicine and the Mercy Hospital Institute of Radiation Therapy.

<sup>1</sup> Schmitz, H. E., Sheehan, J. F., and Towne, J. *Am J Obst & Gynec* 45:377, 1943.

**CASE 1**—A woman aged 46 entered the hospital complaining that she had noted abnormal vaginal bleeding during the three years preceding entry. She had been pregnant five times and had borne 2 children. The fallopian tubes and ovaries had been removed six or seven years before. Microscopic examination of material removed from the uterus by curettage revealed adenocarcinoma of the endometrium (grade 3) (fig. 1). Combined roentgen and radium therapy was instituted. The total dose of radium was about 4,500 milligram hours, the total dose of roentgen radiation was about 4,000 roentgens (r) in the midpelvis. The uterus was removed three months after the completion of the treatment.

The uterus measured 10.4 cm. in length and 7 by 4.5 cm. at the fundus. The peritoneal covering was in part reddish tan and in part bright red and smooth except for several scattered torn red fibrous adhesions. The myometrium was about 2 cm. in thickness and contained one well demarcated mass of shiny white firm (horled) tissue, 1.5 cm. in diameter, in the posterior wall near the left cornu. In the lower portion of the body immediately above the internal os and apparently extending down to involve the os there was a slightly raised mass with a flat, plateau-like top. This mass measured 2.5 cm. in length (in the long axis of the uterus) and completely encircled the lumen. It was composed of rather firm, yellowish white tissue replacing the endometrium and the adjacent myometrium to a depth of 3 mm. Its free aspect was covered by a thin layer of greenish yellow material. The endometrium elsewhere in the body was less than 1 mm. in thickness, smooth and tan or reddish tan.

The cervix was about 4 cm. in length and 3.5 cm. in diameter. The margins of the external os were slightly reddened and granular. Except for an occasional bright red patch the membrane covering the vaginal portion was normal. The cervical canal was lined by a smooth yellowish white membrane. The wall of the cervix was composed of pliable white tissue beneath a thin mucous membrane.

Routine sections were made and the absence of carcinoma established. Later three parallel transverse incisions passing completely through the uterus were made. These divided the uterus into four segments including the upper portion of the body, the lower portion of the body exclusive of the part adjacent to the internal os, the internal os and the portions of the body and of the cervix immediately adjacent, and the remainder of the cervix. Each of these four segments was further divided by longitudinal incisions (in the long axis of the uterus) so placed that in blocks about

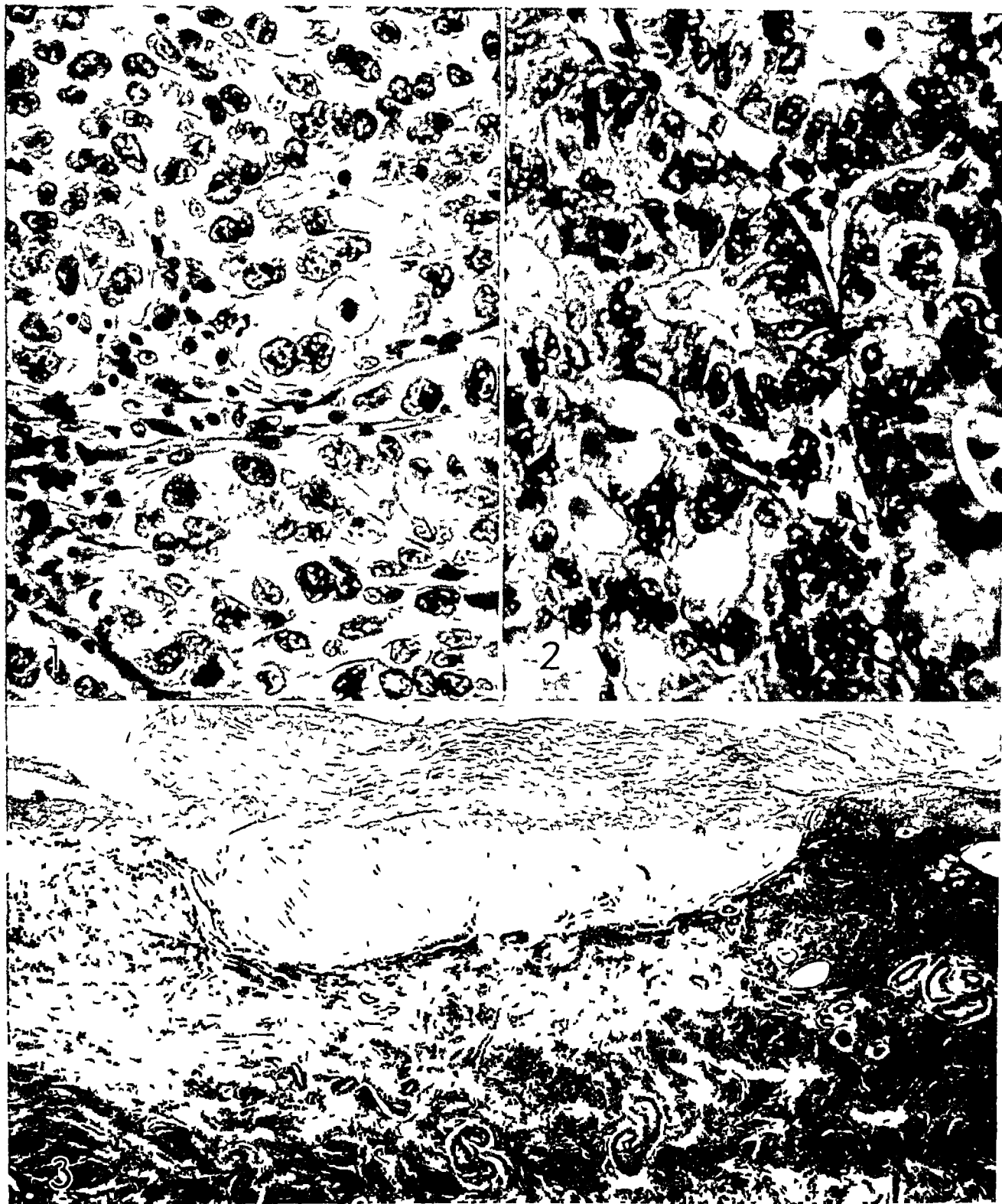


Fig 1 (case 1)—Microscopic appearance of the adenocarcinoma of the endometrium eradicated by radiation therapy. All parts of the tumor included in the sections examined had a similar appearance.  $\times 436$

Fig 2 (case 3)—Microscopic appearance of the adenocarcinoma of the endometrium eradicated by radiation therapy. Most of the tumor was adenomatoid in type. In several areas, however, the pattern resembled that shown in figure 1. The structure of the tumor in cases 2 and 4 was varied. Patterns similar to those shown in figures 1 and 2 were revealed in each instance. In addition, in case 2 distinctly papillary areas were found.  $\times 455$

Fig 3 (case 2)—Typical radionecrotic plaque in the innermost part of the wall of the uterus. The plaque projects slightly into the uterine cavity above. It is well demarcated from the underlying myometrium. Except for changes in small arteries near the plaque, the myometrium is essentially the same adjacent to the plaque as at a distance from it. Note the absence of granulation tissue along the periphery of the plaque.  $\times 16$



4 mm in thickness all of the mucosa and as much as possible of the entire thickness of the wall were included. In the segments of the body of the uterus this was not feasible. Hence only the inner 18 cm of the myometrium appeared in the blocks. Separate sections were made of the serosa and of the outer portion of the myometrium. The blocks were fixed in Bouin's fluid or in 4 per cent solution of formaldehyde. Paraffin sections from the surface of each block were prepared, then each block was cut approximately half way through, and additional sections were made. Thus the entire endometrium and endocervix with the tissues adjacent were sectioned at intervals no greater than 2 mm. The preparations were stained with hematoxylin and eosin. When indicated, Verhoeff's elastic tissue stain and Masson's trichrome stain for connective tissue were utilized.

**Microscopic Examination**—(a) **Upper Body** The endometrium was thin, the glands were few, some were cystically dilated. In some areas the epithelial cells on the surface were low or tall columnar, but for the most part they were cuboidal, with large round vesicular nuclei. Many of these cells had rounded free margins projecting somewhat into the uterine cavity, giving a scalloped appearance to portions of the surface. In some of these rounded cells vacuoles were found, often surrounding a pyknotic nucleus. Over large areas no epithelial covering cells could be seen. The epithelial cells lining the cystically dilated glands were flat or low cuboidal, those lining the more normal glands were for the most part markedly swollen and roughly cuboidal and contained large round or oval vesicular nuclei, the latter sometimes were arranged with long axes parallel to the lumens of the glands. Many of the nuclei were even more irregular. Many contained prominent nucleoli. In a few of the cystically dilated glands hemosiderin-laden macrophages or granular red or brownish red material was noted.

The endometrial stroma was fairly thin even where the endometrium passed outward for a short distance between muscle bundles. Between these prolongations it was particularly thin, often only five fibroblast-like cells thick. In areas in the most superficial portion of the endometrium there was definite edema, the connective tissue cells were flattened, elongated and separated by spaces. Their long axes were arranged parallel to the surface. Deeper the stroma cells were normal in appearance and were not abnormally separated. In a rare area a small irregular hyaline mass intermixed with red cells was noted in the endometrium. The overlying epithelial cells were in part swollen and basophilic, their long axes were parallel to the surface. Near the hyaline deposit the endometrium was edematous and infiltrated by numerous lymphocytes, monocytes and plasma cells, with fewer neutrophils and eosinophils intermixed. Elsewhere the endometrium either showed no cellular infiltration or was infiltrated by a few lymphocytes.

Near the small hyaline mass in the endometrium, some of the muscle bundles of the upper part of the myometrium were partially hyalinized. Among these, clumps of plasma cells and of red cells were found. Elsewhere the muscle bundles of the myometrium appeared normal or somewhat larger than normal. There was no increase in the connective tissue between them. A few small clumps of plasma cells were scattered throughout the myometrium in the section, even deep in it. A few small foci of leiomyoma were encountered, in the smaller ones the neoplasm was cellular, with nuclei closely set, owing to the small amount of cytoplasm

in the individual cells, in the larger ones it was partially hyalinized.

Fairly numerous small arteries (137 microns or less in external diameter) with hyalinized walls were noted in the inner layer of the myometrium near the endometrium or in the lower layers of the endometrium itself. In a few areas in the vascular middle layer of the myometrium larger arteries (500 to 900 microns) showed marked swelling of the internal elastic membrane, the so-called "vitreous change," or an increase in elastic tissue in the adventitia. Sometimes in the relatively wide expanses of altered elastic tissue resulting from the degeneration of old larger arteries, new small arteries were found. These arterial changes have been considered indicative of subinvolution of the uterus (Schwarz<sup>2</sup>).

(b) **Peritoneal Coat** Focal fibrous thickening of the connective tissues of the peritoneum covering the uterus was noted. Torn fibrous adhesions were attached in areas. Refractile material in contact with foreign body giant cells and a few lymphocytes were found in the fibrous tissues in the adhesions as well as in the foci of peritoneal thickening.

(c) **Lower Body** In this segment there was noted a portion of the necrotic mass seen grossly at the internal os and in the adjacent portion of the body. The junction of this mass with the endometrium was also present in this segment. At the time of the gross examination this mass was interpreted as being the carcinoma revealed by curettage or the scar at the former site of a completely destroyed cancer. Microscopically it proved to be an area of coagulation necrosis simulating an infarct. In the descriptions which follow, this mass will be referred to as the plaque.

The central, thickest portion of the plaque consisted of rather finely granular, in areas faintly fibrillary, pale eosinophilic material abutting on the uterine cavity. In it were noted non-nucleated, ghostlike remnants of blood vessels and even more poorly defined outlines of muscle bundles. Some of the arteries were 300 microns or more in diameter. Some contained discrete red cells and were not thrombosed. In the deeper portion of this superficial zone a few neutrophils and many pyknotic muscle cell nuclei were present. This thick necrotic zone rested on altered myometrium. In the latter, two zones could be defined in areas, a deep zone of purely edematous myometrium with beginning atrophy of muscle fibers and a more superficial layer in which the muscle fibers were completely atrophic or necrotic and in which varying amounts of hyaline material were deposited. These two zones will be designated as the edematous myometrial zone and the hyaline-edematous myometrial zone respectively. Each was about 0.5 to 1 mm in thickness. The two zones were not always sharply separated. They were best defined in the sections from material fixed in solution of formaldehyde in contradistinction to those preserved in Bouin's fixative.

The hyaline-edematous zone was usually, but not always, rather sharply marked off from the overlying zone of necrosis. In the hyaline-edematous zone only the nuclei of muscle cells persisted. These were often found in vacuole-like spaces. Around the blood vessels in this zone, and even around the vacuoles containing the nuclear remnants of muscle cells, irregularly distributed, faintly fibrillary sheets of hyaline material were deposited. In a few areas the deposits were dense enough to obliterate vacuoles and nuclei. Scattered giant fibroblasts and endothelial cells were present in

the hyaline-edematous myometrial zone and less often in the edematous myometrial zone. Because of the disappearance of muscle bundles and the persistence of blood vessels, a false impression of a zone of granulation tissue in the more superficial portion of the hyaline-edematous myometrial zone was obtained. Close scrutiny indicated that there was no new formation of blood vessels. In fact, in many areas there was no suggestion of granulation tissue. There was a well defined transition from normal myometrium to edematous myometrium, from the latter to a partially hyalinized, edematous necrobiotic myometrium and finally from this hyaline-edematous portion to completely necrotic tissue. (Compare fig. 8 in a previous article<sup>3</sup>) Over large areas there was no cellular infiltration in any of these four layers. Here and there in the hyaline-edematous myometrial zone (and even in the lower part of the completely necrotic layer) masses of red cells were seen. In the same areas there were deposits of hemosiderin or of hemosiderin-laden macrophages, sometimes accompanied by lymphocytes, monocytes and even eosinophils. Over the free surface of this portion of the plaque no epithelium was encountered, but there was a sharp line of demarcation from the lumen. At the junction of the plaque with the endometrium, however, the surface epithelium continued up over the plaque for a short distance. The epithelial cells here were swollen and basophilic, their nuclei were swollen and hyperchromatic, with the long axes parallel to the surface. A few neutrophils were noted in the plaque just beneath the desquamating epithelial cells. Near its junction with the endometrium the plaque was composed in areas of fused red cells, intermixed with neutrophils and fibrin, which penetrated beneath the surface epithelium of the adjacent endometrium and near the endometrium rested directly on the hyaline-edematous myometrial layer. Farther away from the junction of plaque and endometrium, as the thicker portion of the plaque was approached, the typical infarct-like necrotic surface layer replaced the fused red cell material. This, however, in places still remained superficial to the infarct-like zone of necrosis. Near the junction of the plaque with the endometrium in the hyaline-edematous myometrial zone, hyalinized necrotic muscle fibers, darker red than the other form of hyalin adjacent to them, were noted. Definitely identifiable hyalinized muscle fibers were noted only here in the margin of the plaque. Elsewhere the hyaline material could not be definitely traced to altered muscle cells but seemed rather to have been deposited there from the blood stream or to be altered blood clot.

Neither endometrium nor any identifiable portion of it appeared in the plaque except at the margin where the material of the plaque was continuous with similar material in the endometrium and where the surface epithelium continued from the endometrium up over part of the plaque. Elsewhere the endometrium was histologically similar to the endometrium in the upper portion of the body of the uterus.

The changes in the myometrium in contact with the plaque have already been described. Immediately beneath the intact endometrium there was mild edema of the myometrium. Occasionally a few monocytes were noted here. Deep in the myometrium beneath the plaque a few small clumps of lymphocytes and monocytes were situated near blood vessels or lymphatics. A few small foci of leiomyoma (0.1 to 1 cm. in diameter) were seen in this portion of the myo-

metrium. In the larger ones, varying degrees of hyalinization and of early cystic change were observed. In the smaller ones, central hyalinization was not uncommon.

Marked changes were present in the arteries in the edematous and hyaline-edematous zones of the altered myometrium beneath the plaque. These changes consisted of swelling of endothelial cells, often with intimal edema and an infiltrate of lymphocytes, monocytes and even neutrophils and eosinophils, the presence in the intima of red cells, discrete or fused, of foam cell plaques<sup>3</sup> and of fibrin deposits, necrosis of portions of the vessel walls, particularly of the inner portions, and the presence of thrombi. The arteries involved measured 300 microns or more in diameter. The size of the involved arteries indicated that the plaque extended fairly deeply into the myometrium and possibly explained why hyaline intimal thickening and complete hyalinization of the arterial walls, well known effects of radiation treatment, were not common here. In fact, these hyaline changes were seen only in the arterioles in the innermost layer of the myometrium beneath the edge of the plaque.

Several myometrial arteries with long axes perpendicular to the surface were found running parallel to one another. As they passed through the edematous and hyaline-edematous myometrial zones into the zone of complete necrosis, the changes listed in the foregoing paragraph occurred and were largely limited to the portions traversing the altered myometrial zones or the tissues immediately adjacent. These changes were quite similar in the various arteries and were most pronounced near the zone of necrosis.

(d) Internal Os. The plaque was found throughout this segment, which included the portions of the body and of the cervix immediately adjacent to the internal os. An excellent opportunity was afforded to contrast the changes in the plaque and the adjacent tissues near the cervical termination of the plaque with those occurring in the uterine body near the upper termination of the plaque. Toward the uterine body the structure of the plaque and of the two subjacent altered myometrial zones and the type and the location of the arterial changes were the same as those reported in the description of the lower portion of the body of this uterus.

Toward the cervical side the hyaline-edematous zone was much more compact—in areas, in fact, not edematous at all. Superficial to it in areas was a granular necrotic zone, surmounted in turn by a layer of old blood clot. In the hyaline zone near the cervical termination of the plaque was a cervical gland with swollen, basophilic, poorly outlined, desquamating cells. Most of the nuclei in the gland were pyknotic.

Deep to this fairly dense hyaline zone, instead of a fairly wide edematous zone to correspond with the edematous myometrial zone there was a narrow zone of partially hyalinized fibromuscular tissue. In some of the slides this zone was continuous toward the cervical side with an area of loose structure filled with masses of red cells and hemosiderin, free or in macrophages. Plasma cells were abundant in the same area. Portions of dilated, partly necrotic arteries were seen in this same area. Some were filled with partially organized clot of an orange color, others contained discrete red cells. Hemorrhage undercut part of the endocervix at the lower margin of the plaque. There were masses of red cells and of plasma cells here. Elsewhere the endocervix was mildly edematous and infiltrated by a few lymphocytes. The glands in it

near the plaque were composed of swollen, basophilic cuboidal cells with large round nuclei and did not secrete mucus. On the surface were a few cuboidal epithelial cells.

The edge of the plaque extended beneath the surface epithelium of the cervical canal in areas at least. The surface epithelium extending up over the edge of the plaque was composed of club-shaped cells, the free margins of which were ballooned out. Deep down in the wall of the cervix, extending from the area of recent and old hemorrhage, were clumps of plasma cells and of hemosiderin-laden macrophages, probably in or around lymphatics.

In contrast to the changes noted in arteries near the upper termination of the plaque in the body of the uterus, hyaline intimal thickening with or without occlusion and hyalinization of vessel walls were much more prominent in the cervical tissues immediately adjacent to the plaque and particularly near its lower edge. Here masses of hyalinized arteries were noted. Most of these were less than 300 or 350 microns in diameter. In addition, deeper in the wall of the cervix were notable vascular changes: dilated arteries with necrosis of parts of their walls, foam cell plaques, fibrin deposits in the intima, even hemosiderin and hyaline material in the intima. Occasionally fibroblastic intimal thickening was present. Some small arteries (about 100 microns) had completely hyalinized necrotic walls. The adventitia was edematous and infiltrated by lymphocytes, monocytes and occasionally by other cells as well.

(e) *Cervix*—The stratified squamous epithelium was absent in areas near the external os. Beneath one such denuded area there was hyalinization of a small portion of the fibromuscular coat. Masses of red cells, lymphocytes and monocytes infiltrated this area, and pronounced vascular changes, including necrosis of dilated arteries and hyaline necrosis of walls of other, undilated arteries, occurred.

Beneath the stratified squamous epithelium, as well as beneath the columnar epithelium, were well defined lymphoid follicles and, in areas, massive diffuse infiltration by lymphocytes and plasma cells. Fewer neutrophils and eosinophils were present. The epithelial cells of some of the cervical glands had swollen pale nuclei, others contained pyknotic nuclei. The fibromuscular coat was definitely not diffusely hyalinized.

*Summary*—The observations included small intramural foci of leiomyoma, atrophy of the endometrium, focal chronic endometritis, radionecrosis of part of the wall of the uterus at the level of the internal os, marked radiation changes in arteries near the area of necrosis, mild chronic metritis, marked chronic cervicitis, focal chronic perimetritis with foreign body giant cell reaction, no evidence of carcinoma.

*CASE 2*—The patient, aged 52, complained of vaginal bleeding during the six months preceding admission to the hospital. There had been three normal deliveries and no miscarriages. The menopause had occurred eight years before, and there had been no vaginal bleeding since until the onset of the present illness. Microscopic examination of uterine curettings revealed papillary adenocarcinoma of the endometrium (grade 2). Combined roentgen and radium therapy was instituted (6,000 milligram hours of radium and 4,000 r of roentgen radiation in the midpelvis). About five months after the completion of this treatment the uterus was removed.

The uterus measured 7.8 cm in length and 4.8 by 3 cm at the fundus. The serosa was smooth and

glistening, its color was pinkish tan. The myometrium was about 1.4 cm thick and composed of fibrillary, pale grayish tan tissue. At the level of the internal os there was an irregular mass on the mucosal aspect, 0.8 cm in diameter, extending 0.5 cm above the surrounding mucosa. This plaque was composed of soft grayish brown or pale greenish brown tissue. There was no gross extension of this tissue into the myometrium or the adjacent pale grayish white tissues of the cervical wall. Projecting into the uterine cavity from the endometrium of the fundal portion was a soft polypoid mass of pale grayish brown tissue, measuring 1.1 by 0.8 by 0.4 cm. Elsewhere the endometrium was pale pinkish tan and 1.6 mm thick. The cervix measured 3.6 cm in length and 3.4 cm in diameter. The external os was mildly patulous, its margins, irregularly stellate. The mucous membrane of the vaginal aspect was smooth and pinkish gray except at the external os, where it was finely granular and pinkish tan.

The body of the uterus was divided longitudinally into two unequal halves. Microscopic examination of sections taken at that time revealed no carcinoma. Later the smaller unequal half was cut into numerous blocks by parallel incisions passing obliquely through the entire wall. The cuts were so spaced that all of the endometrium was contained on the 4 mm thick edges of the blocks. Sections from this portion were labeled "½ Body." The rest of the uterus was divided into three transverse slabs labeled "Upper Body," "Lower Body" and "Cervix." These were later subdivided longitudinally and sections obtained in a manner similar to that in which sections were prepared in case 1.

*Microscopic Examination*—The changes observed in the slides labeled "½ Body" and "Upper Body" were similar and will be described together.

(a) *One-Half Body: Upper Body*—The endometrium resembled that of the uterus in case 1. There was definite atrophy. The glands were reduced in number and widely separated. Some were cystically dilated. In the more superficial portion of the endometrium the well preserved fibroblast-like stromal cells were separated somewhat (mild edema). The epithelium in the glands and on the surface resembled that in case 1, but the constituent cells were not so swollen. Some of the cystically dilated glands contained red blood cells or hyaline globules. Some of the latter were large and resembled corpora amylacea. There were in areas a few lymphocytes, plasma cells, monocytes and red cells.

The inner half of the myometrium seemed much more compact than the outer half. In the inner half the muscle cell nuclei were much closer together than normally. This appearance was interpreted as indicating atrophy of these cells. At least the cytoplasm was reduced in volume. In the outer half there was considerable separation of the muscle and connective tissue elements, probably signifying edema. In the outer half, just beneath or extending into the serosa were a few calcospheroids and a few small glandlike spaces lined by mesothelium or epithelium. No endometrial stroma was noted around these glandlike structures. The serous coat was otherwise normal. A rare clump of lymphocytes was seen deep in the myometrium.

(b) *Lower Body*—In the middle coat of the myometrium, arterial changes characteristic of subinvolution (compare case 1) were observed. Near the endometrium there were only a few hyalinized, unoccluded arterioles.

In some sections a plaque similar to that in case 1 was in evidence (fig 3). It was composed of a fairly wide zone of necrotic tissue in which the faint pattern of blood vessels, of muscle bundles and even of a few glands with swollen epithelium was still visible, particularly in the deeper portions. Masses of red cells were present in some parts. The necrotic zone was, however, much more compact, more hyaline-appearing than the corresponding zone in case 1. The necrotic layer was rather sharply demarcated from the underlying myometrium. No definite partially hyalinized, edematous myometrial zone was noted. In areas the necrotic material rested directly on apparently normal myometrium or myometrium in which only a few hyalinized muscle fibers could be seen. Elsewhere a narrow zone of edematous myometrium containing partially atrophied muscle fibers was present. Bizarre fibroblasts, few in number, were located in the lower part of the necrotic layer rather than in the edematous myometrial zone. The latter was infiltrated in a few areas by small numbers of lymphocytes, neutrophils, hemosiderin-laden macrophages, monocytes and eosinophils. A few small calcospheroids were also noted in the myometrium at the margin of the plaque. There were areas at the junction of the myometrium and the plaque where no cellular infiltration could be found.

Superficial to the infarct-like layer were masses of fused red cells (old hyalinized clot) with a few neutrophils and large calcified globules. The plaque extended into the adjacent endometrium at its margin. The surface epithelium of the latter extended up over the edge of the plaque. The epithelial cells in this extension were swollen and contained fairly large pyknotic or hyperchromatic elongated nuclei, the long axes of which were parallel to the surface.

Where the plaque met the endometrium a few lymphocytes and hemosiderin-laden macrophages were present in the endometrium.

In the myometrium adjacent to the plaque there were vascular changes. These consisted predominantly of hyalinization and thickening of the intima. In some arteries the media was swollen, hyalinized and even infiltrated by neutrophils. A few had swollen endothelial cells with or without lymphocytes and foam cells in the intima. Some arteries showed red cells in their walls. Rarely, mild fibroblastic intimal thickening was encountered. Deep in the myometrium a few small clumps of lymphocytes appeared in contact with the walls of veins or the lymphatics.

(c) *Cervix*—The plaque appeared in these sections also. Here, too, it was compact, rather densely hyaline but with a suggestion of whorled muscle bundles in its deeper part. A few necrotic blood vessels with discrete red cells in the lumens were found in it. The plaque was rather sharply demarcated from the adjacent viable fibromuscular tissue, which showed little if any hyalinization. In parts of the base of the plaque and in the adjacent fibromuscular tissues there were clumps of lymphocytes and hemosiderin. Elsewhere no cells were noted adjacent to the plaque.

Near the plaque the arteries showed mainly hyaline intimal thickening and varying degrees of hyalinization of the medial coat. Farther from the plaque, even deep down in the fibromuscular coat, arterial changes, much more varied, were seen, such as dilatation and necrosis, subendothelial presence of lymphocytes and fibrin, presence of foam cell plaques and intimal hyalinization. Clumps of lymphocytes, monocytes and eosinophils were found near blood vessels throughout the wall of the cervix.

Beneath the edge of the plaque cervical glands were identified. No cellular infiltration was seen in the poorly defined adjacent endocervical stroma. Some of the cervical glands at the edge of the plaque were dilated, contained thick mucus and were lined by flattened epithelium. Other dilated glands were lined by low columnar epithelium. Most of the surface columnar epithelium was replaced by a thin layer of fibrin and red cells. No other cells were found with the red cells.

*Summary*—The observations included an area of radionecrosis at the internal os with vascular changes in adjacent tissues, atrophy of the endometrium, partial atrophy of the inner half of the myometrium, mild chronic metritis, a completely necrotic polyp in the body of the uterus with a rare identifiable epithelial cell in it, chronic cervicitis, no evidence of carcinoma.

*CASE 3*—A woman aged 38 complained of vaginal bleeding, menorrhagia of one and a half years' duration, metrorrhagia of ten months' duration and severe hemorrhage occurring seventeen days before. She had been pregnant twice and had two children. By curettage a diagnosis of adenocarcinoma of the endometrium (grade 3) was established (fig 2). A total dose of roentgen radiation of 4,000 r in the midpelvis was given in divided doses, along with a total dose of radium of 6,000 milligram hours. About eight months after completion of this therapy the uterus, tubes and ovaries were excised.

The uterus measured 10.7 cm in length and 7.5 by 5.5 cm at the fundus. The serosa was pinkish tan, smooth and glistening. The myometrium was 3.2 cm in thickness, fibrillary and pale grayish tan. In the lower portion of the body was a plaque of soft, yellowish gray to yellowish tan tissue extending completely around the lumen. It rose about 0.4 cm above the surrounding endometrium. The plaque was 3.3 cm in length (in the long axis of the uterus). It extended downward into the upper part of the cervical canal. It extended outward into the wall of the uterus to a depth of no more than 0.5 cm. It was well demarcated from the myometrium.

The cervix measured 3.5 cm in length and was 3 cm in diameter, roughly globoid. The cervical os was patulous, its margins, stellate.

The uterus was cut into a multitude of blocks. The method of obtaining the blocks and the final individual slides has already been described in this paper (case 1). Just as in case 1, the entire thickness of the myometrium of the body could not be included in the same section with the endometrium, only the inner 1.5 cm of the myometrium was retained with the endometrium. Separate sections of the serosa and the outermost portion of the myometrium were prepared.

*Microscopic Examination*—(a) *Upper Body*—The endometrium was thin and contained few glands. The connective tissue cells of the lamina propria were thin and elongated, with long axes parallel to the surface. They were separated by spaces (edema), particularly in the more superficial portion of the mucosa. The endometrium was infiltrated by plasma cells with fewer monocytes, lymphocytes, eosinophils and hemosiderin-laden macrophages. In areas masses of plasma cells, often alongside masses of red cells, were seen. The few glands present were lined by flattened epithelium. Much of the surface epithelium was absent. Columnar epithelial cells and swollen cuboidal cells predominated in the areas still covered by epithelium. In the large cuboidal cells there were vacuoles, often con-

taining linearly compressed nuclei with long axes perpendicular to the surface. In areas some islands of stratified squamous epithelium with spines but without cornification were identified. In other areas low cuboidal cells with deeply basophilic elongated nuclei with long axes parallel to the surface were present. In a few areas the free borders of the surface epithelial cells were convex toward the lumen, giving a scalloped appearance to the free surface of the epithelium.

Except near the endometrium, the myometrium was composed of normal or possibly of somewhat enlarged muscle fibers, arranged in bundles. Between these there was a moderate increase in the amount of connective tissue. Some of the muscle fibers near the endometrium were mildly hyalinized and separated a little from one another by small spaces (edema?). In a rare area there was marked hyalinization of some of the muscle bundles near the endometrium. Here considerable plasma cell infiltration was noted. Clumps of plasma cells were also seen somewhat deeper in the myometrium.

There was complete hyalinization of many of the small arteries (100 microns or less in diameter) in the myometrium near the endometrium. Some of these were occluded. In larger arteries, only the internal layers of the wall were hyalinized.

The serosa was essentially normal.

(b) Lower Body The plaque noted during the gross examination was found in all of the section of this segment of the uterus. No endometrium was present.

The plaque was composed over large areas of a fairly thin surface layer of fibrin and neutrophils and immediately subjacent, a layer of old hyalinized blood clot (or fused red cells). Deep to this lay a zone of partially hyalinized, edematous, necrobiotic myometrium (fig 4) and finally, still deeper, a zone of edematous myometrium with some degree of atrophy of the individual muscle cells. The last two zones were 0.5 to 1 mm thick respectively. In the hyaline-edematous zone near the old clot giant bizarre fibroblasts and endothelial cells were so prominent as to give the impression of definite granulation tissue extending into the clot (fig 4). There did seem to be some increase in the number of capillaries here, and so it is probable that true granulation tissue was present. The hyaline material in this zone was paler than that in the old clot adjacent and seemed to be deposited along capillaries in particular, as well as along individual necrotic muscle fibers. It was difficult to decide whether or not this hyalin was not part of the denser old hyaline clot adjacent to it. Sometimes in this zone definitely hyalinized, not swollen, muscle cells with fading nuclei were seen but they were not numerous.

The surface clot was present throughout all of the sections. There were no ghosts of former endometrial or myometrial structures in the surface necrotic layers.

For the most part there were no inflammatory cells in the edematous myometrial zone or in the lower part of the hyaline-edematous zone. In the upper part of the latter zone, however, there was usually mild to moderate infiltration by lymphocytes and monocytes, with fewer neutrophils, eosinophils and hemosiderin-laden macrophages (fig 4). Red cells in masses were not uncommon in this zone. In a few parts of this zone masses of plasma cells and of hemosiderin occurred and seemed to overflow into the adjacent edematous myometrial zone or even into the neighboring more normal myometrium.

The fibroblasts and even the endothelial cells of the capillaries in the upper part of the hyaline-edematous zone were most bizarre, they were spindle shaped, triangular, stellate or even more irregular, they had dark

cytoplasm and giant nuclei, single or multiple. The giant nuclei were often hyperchromatic, sometimes pyknotic, appearing as dark basophilic smudges, at times even coarsely vacuolated. The cytoplasm of the same cells in some instances contained vacuoles or even neutrophils.

Down through the myometrium beneath the plaque, clumps of lymphocytes and monocytes were observed near vascular channels.

Vascular changes were most prominent in the edematous myometrial zone and consisted mainly of complete or partial hyalinization of the walls of very small arteries (100 microns or less), sometimes with occlusion, and intimal hyalinization in larger arteries. In the hyaline material in some of the hyalinized vessels pale areas or vacuoles could be seen.

(c) Internal Os The plaque appeared also in the sections from this segment of the uterus. For the most part, the tissues beneath it were composed of the same layers as in the lower body segment. Toward the cervix, however, the hyaline-edematous zone became more hyaline and compact, and the subjacent edematous zone disappeared. In a few areas the layer of fibrin and neutrophils on the surface dipped down into the layer of old hyalinized clot, replacing varying thicknesses of it and in at least one area replacing the entire layer of clot and even the uppermost part of the hyaline-edematous zone. Where this event occurred there were in the hyaline zone definitely hyalinized muscle fibers, much more numerous than were seen in any other area. Some of these hyaline muscle fibers had faintly staining nuclei and appeared somewhat like partially autolyzed fibers.

A second change was noted in this same area: fibrinoid necrosis of walls of small blood vessels. In other words, the walls seemed to be composed entirely of fibrin or fibrin-like material, internal to which in areas swollen endothelial cells could be seen. Discrete red cells were present in some of these vessels, clots, in others. Toward the body of the uterus the arterial changes were most pronounced in the edematous myometrial zone and consisted mainly of hyalinization. Toward the cervix, even though still in the myometrium, the arterial changes were more diversified. Dilated, partially necrotic arteries with hemosiderin around them were noted in the edematous myometrial zone or on either side of it. Massive plasma cell infiltration occurred here, along with the hemosiderin. Other changes included hyaline deposits in the intima, fibrin, or lymphocytes, monocytes and other cells in the intima, foam cell intimal plaques and even hyaline deposits mixed with hemosiderin in the intima. Where fibrin and neutrophils replaced the layer of old clot and the upper part of the hyaline-edematous zone, fibrinoid necrosis of blood vessels ensued in the hyaline zone, and the changes already described in the larger arteries occurred in the subjacent tissues. In general, toward the cervical side arterial changes were located at greater distances from the necrotic plaque than they were on the uterine body side.

Throughout the wall of the cervix, adjacent to the plaque, small clumps of cells, mainly plasma cells, could be found along the course of blood vessels. In the myometrium the clumps were composed predominantly of lymphocytes.

The portions of endocervix seen in these sections were covered by a layer of stratified squamous epithelium instead of columnar. Some of the cervical glands were lined by columnar, mucin-secreting cells, others, by swollen columnar cells which did not secrete mucus.

(d) Cervix There was no evidence of a plaque in any of the sections labeled "Cervix." However, the canal was lined by stratified squamous epithelium, beneath which accumulations of plasma cells and lymphocytes





FIGURES 4, 5 AND 6  
(See legends on opposite page)

with a few neutrophils and eosinophils could be found. Where the stratified squamous epithelium was particularly thin, well defined lymphoid follicles had formed. In areas beneath the stratified squamous epithelium covering the vaginal aspect, some focal hyalinization of the fibromuscular coat and accumulations of red cells were found.

There was no hyalinization of the connective tissues around the cervical glands. Some of the latter were lined by columnar, mucus-secreting cells, others, by swollen cuboidal epithelial cells with large nuclei. A few glands were cystically dilated.

Toward the upper end of the portions of the cervical canal which appeared in these sections numerous arteries showed the effect of radiation and were similar to those described under the heading "Internal Os." Even thrombosed arteries were encountered. Along the blood vessels or lymphatics in the wall of the cervix were clumps of plasma cells with lymphocytes or monocytes or both intermixed.

**Summary**—The observations included an area of radionecrosis at the level of the internal os with marked vascular changes in the adjacent tissues, atrophy of the endometrium, focal squamous metaplasia of the columnar epithelium of the endometrium, chronic endometritis, chronic metritis, diffuse fibrosis of the myometrium, possibly mild muscular hypertrophy of the body of the uterus, marked chronic cervicitis, bilateral chronic salpingo-oophoritis and perisalpingo-oophoritis, small left hydrosalpinx, atrophy of the ovaries, no evidence of carcinoma.

**CASE 4**—An unmarried woman, aged 65, consulted her physician because of an odorless, watery, at times blood-tinged vaginal discharge which had been present for three months. The menopause had occurred twelve years before. The curettement revealed adenocarcinoma of the endometrium (grade 2). The standard radiation therapy was given—radium, 6,000 milligram hours, roentgen radiation, 4,000 r, in the midpelvis. A hysterectomy was made about four months after the therapy.

The uterus was received in five pieces. The largest of these measured 53 by 36 by 25 cm and apparently comprised the greater portion of the body. Another piece, measuring 45 by 3 by 16 cm, included all of the cervix. The external os was widely dilated, the margins were thin, dark grayish red or reddish tan. The cervical canal was lined by friable, dark greenish gray or reddish gray material, 1 mm thick. Where identified, the serosa over the body was pinkish tan and smooth. Over large areas it was absent, and shaggy torn.

myometrium was exposed. The endometrium in the body was rough, somewhat shaggy, greenish gray to reddish gray and thickened in one area, where it measured 0.6 cm in diameter. The myometrium was pale grayish tan, fibrillary and 1.4 cm in thickness. In it several firm, well demarcated masses of glistening, grayish white whorled tissue were found. A mass of similar tissue, 2 by 1.5 by 1 cm, was received separately. The central portion of this mass was calcified. Finally, a separate strip of peritoneum and smooth muscle, apparently from a cornu of the uterus, was also received.

The largest portion of the uterus received, apparently the major portion of the body, was cut into two transverse slabs, each of which was divided by numerous longitudinal incisions into blocks about 4 mm thick, which included the entire wall. Since it was impossible to be certain which portion of the mass was the fundus and thus to orient the slabs, the sections from them were labeled "Body 1" and "Body 2" respectively. The cervix was divided by longitudinal incisions into blocks which included the entire wall. Slides were made from these blocks in the same manner as in the preceding cases.

**Microscopic Examination**—(a) *Body 2*. A plaque, somewhat similar to those described in the previous cases, was found in the sections from this segment. The most superficial layer was made up of old hyalinized clot with many neutrophils. In areas much fibrin was intermixed. In the myometrium beneath this layer a fairly wide hyaline-edematous zone, not sharply demarcated from an underlying edematous zone, was seen. In the hyaline-edematous zone and lower portion of the adjacent clot, fibrinoid necrosis of small vessels was apparent. In this and the adjacent edematous myometrial zone there were areas of only mild infiltration by lymphocytes, monocytes, neutrophils, plasma cells and eosinophils. Elsewhere the infiltration by these cells was much more pronounced, and considerable hemosiderin, free or in macrophages, and numerous red cells were often intermixed. In some areas beneath the surface clot a thin zone of edematous myometrium and atrophic muscle fibers were particularly heavily infiltrated by these cells. Here also several bizarre giant fibroblasts were seen. In the areas where a definite hyaline-edematous zone was in evidence, definite transitions from normal muscle fibers to thin atrophic muscle fibers to naked muscle cell nuclei could be traced. As the stage of naked nuclei was approached, there was increasingly larger deposits of hyalin around them. This hyalin was pale in contrast to the darker red hyaline material in the adjacent clot.

#### EXPLANATION OF FIGURES 4, 5 AND 6

**Fig 4 (case 3)**—Hyaline-edematous myometrial zone (pale lower part of field) at the junction of the myometrium with the plaque. The infarct-like area of necrosis of the typical plaque (fig 3) is replaced by masses of fused red cells or old clot (the dark material in the upper part of the field, particularly the right upper corner). Note the pale hyaline material around blood vessels and along necrobiotic muscle fibers in the hyaline-edematous zone. There is a definite suggestion of granulation tissue here and mild infiltration, the infiltrating cells being predominantly lymphocytes and monocytes.  $\times 205$ .

**Fig 5 (case 4)**—Small arteries deep in the wall of the cervix. In the artery to the left a somewhat folded loop of fibrin (dark band) surrounds the narrowed lumen. There are edema and deposition of hyalin in the intima and the inner part of the media with a few neutrophils in the hyaline material. There are large deposits of hyalin in the intima and the media of the artery in the right half of the field. There are no identifiable endothelial cells around the narrow lumen.

**Fig 6 (case 6)**—Necrosis of the superficial portion of the endometrium with deposition of fibrin and massive neutrophilic infiltration. The space in the right upper corner represents the uterine cavity. The stroma of the deeper portion of the endometrium, around the bases of the glands, is edematous and infiltrated by lymphocytes, plasma cells and monocytes as well as by neutrophils. Note the radiation changes in the epithelial cells of the endometrial glands (nontumorous). A small portion of the myometrium appears in the lower left corner of the field. The muscle fibers show little deviation from the normal.  $\times 156$ .



The endometrium throughout the body, even at a distance from the plaque, was completely replaced by a layer of neutrophils and old hyalinized blood clot except in a rare small area. Sometimes the innermost portion of the myometrium was likewise replaced by this material. In the tissues adjacent to this necrotic surface layer were numerous plasma cells, monocytes and hemosiderin-laden macrophages, as well as hemosiderin deposits. In some spots the cellular infiltration and the deposits were massive. The rare island of surface endometrium identified was thin and showed loss of much of the surface epithelium. A few groups of mildly swollen, pseudostratified columnar cells were still attached. Only a rare gland was seen. These were cystically dilated and lined by flattened epithelium. The stroma was composed of attenuated, elongated connective tissue cells, rather widely separated (edema). The stroma was diffusely infiltrated by lymphocytes and plasma cells. Numerous red cells were present

nuclei. Other glands were cystically dilated and lined by flattened epithelium. Some of the glands contained monocytes and lymphocytes or monocytes and red cells in their lumens. The stroma of the ectopic endometrial tissue was in some areas compact in others edematous or somewhat atrophic but not fibrotic. In the stroma in some of the islands of ectopic tissue plasma cells and hemosiderin were encountered. In the myometrium beneath the plaque small clumps of lymphocytes were located along lymphatics or blood vessels. In the upper edematous, partially hyalinized myometrial zone and in the surface zone of complete necrosis with old hemorrhage, fibrinoid necrosis of small blood vessels occurred. Deeper even in the more normal myometrium, but more often in or near the edematous myometrial zone were a few hyalinized small arteries and also arteries with cells and fibrin or foam cell plaques in the intima. There was only a rare hyalinized arteriole near the endometrium.

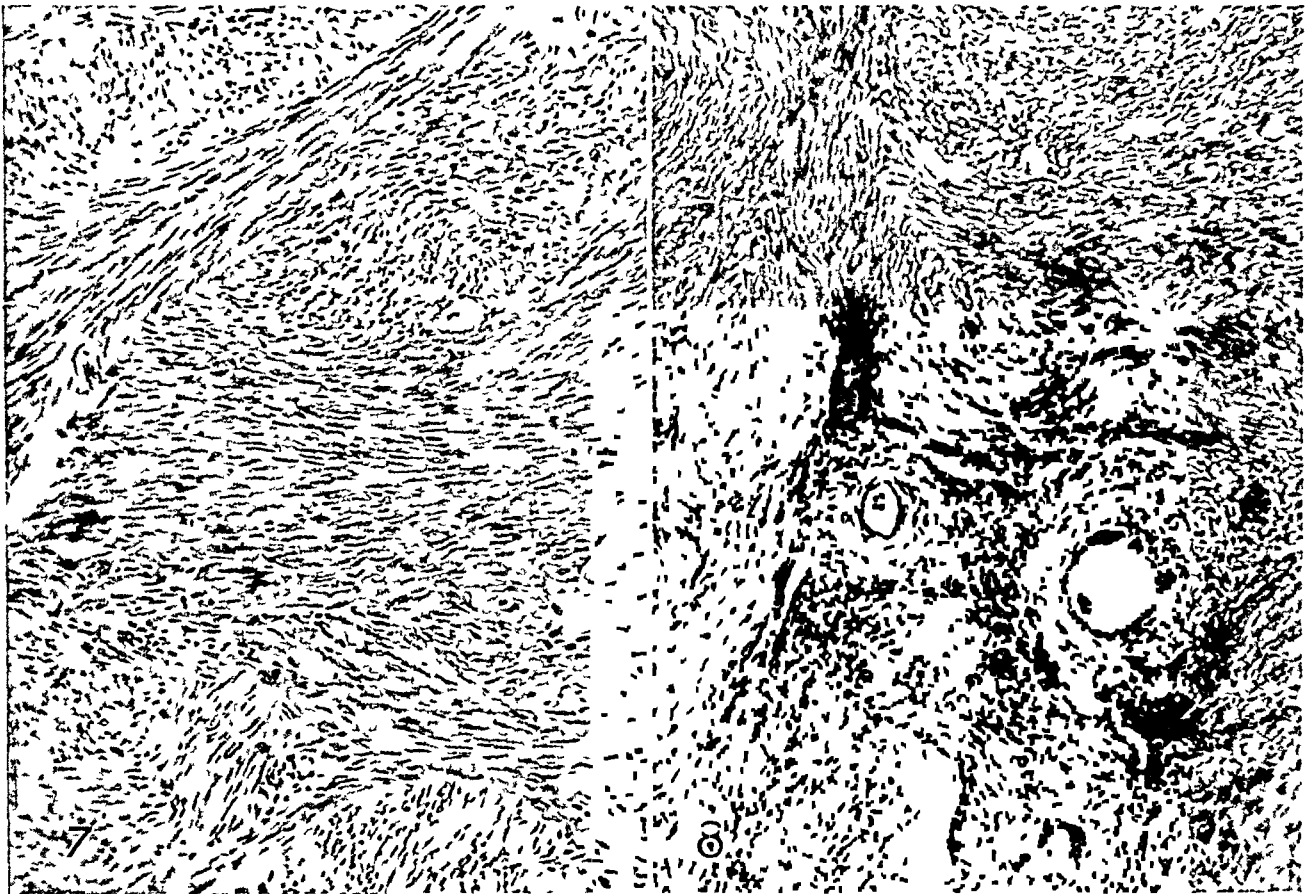


Fig 7 (case 1)—Representative section of the myometrium. There is no muscular atrophy. In fact, the muscle fibers in the section from which this photomicrograph was made seemed somewhat larger than normal.  $\times 125$

Fig 8 (case 4)—Atrophic ectopic endometrium in the myometrium (in the lower half of the field) twelve years after the menopause and four months after the completion of radiation therapy.  $\times 125$

The muscle bundles of the myometrium were of normal size. The individual muscle cells showed little deviation from the normal except near the endometrium or near the surface necrotic layer, where hyalinization and even some gradual necrosis occurred. Here there was infiltration by the cells already previously listed, particularly by plasma cells. In the myometrium small foci of leiomyoma were seen, some partially hyalinized. In addition, numerous islands of ectopic endometrial tissue were identified beneath the plaque and elsewhere as well (fig 8). Those beneath the plaque were composed predominantly of endometrial stroma. However, a few glands were seen in this location. These contained swollen cuboidal epithelial cells with large vesicular

(b) Body 1. Another portion of the plaque just described appeared in the sections from this segment of the uterus. Much of the plaque had the same composition as the portion described already. However, in one area the whole top of the plaque appeared autolyzed and was teeming with bacteria. Beneath this bacteria-laden layer, a mass of fibrin and neutrophils rested directly on a wide zone of markedly edematous myometrium, which extended almost to the serosa. There was massive diffuse neutrophilic infiltration of almost the entire uterine wall here. In that part of this edematous myometrium near the layer of neutrophils and fibrin, definite groups of hyaline muscle fibers or muscle fibers showing gradual attenuation could be

observed. Vascular changes in this region were not notable. Those which occurred consisted mainly of edema of the entire arterial wall with mild infiltration by neutrophils, particularly in the intima. A few arteries at the edge of the plaque were thrombosed. Toward the cervix, arteries with hyaline intimal thickening could be found but even here there were a few neutrophils in the walls. In general, greater neutrophilic infiltration was found in arteries here. Fibrin and red cells were noted in the outer myometrium near the serosa in one region and clumps of lymphocytes and plasma cells near it in other areas. No fibrin was seen on the serosa.

(c) *Cervix*. No mucous membrane was identified anywhere, it was replaced by a layer of necrotic, amorphous debris and fused red cells or old hyalinized clot. This passed directly over into the fibromuscular layer. The latter near the surface was hyalinized in spots and rather massively infiltrated by monocytes, lymphocytes and plasma cells with fewer neutrophils and eosinophils. Small clumps of plasma cells were found deep in the cervix.

Vascular changes were marked, particularly near the upper end of the canal. Fibrin and cells appeared in the thickened intima of some arteries, hyaline necrosis and neutrophilic infiltration of the walls took place in others. Thrombosis was not uncommon. Acute diffuse inflammation of the walls of small veins was also seen.

*Summary*—The observations included acute gangrenous endometritis and endocervicitis with extension of the inflammatory process into the adjacent myometrium and the fibromuscular coat of the cervix in areas, a plaque of radionecrosis at the level of the internal os with secondary bacterial invasion, chronic metritis and cervicitis, acute diffuse metritis near the internal os, radiation changes in blood vessels near the plaque, multiple small intramural foci of leiomyoma, ectopic endometrium in the myometrium, no evidence of carcinoma.

**CASE 5**—A patient aged 50 entered the hospital complaining of a thick white vaginal discharge and of vaginal bleeding. The white discharge had been present for one month, the bleeding, for ten days. Menstruation had ceased five or six years previously, and since that time there had been no vaginal bleeding until ten days before entry. The patient had five children. There had been no miscarriages. The uterus was curetted and a diagnosis of adenocarcinoma of the endometrium (grade 2) established. Radiation therapy included both radium and roentgen radiation (radium, total dose, 6,000 milligram hours, roentgen radiation, 4,000 r in the midpelvis). The uterus, tubes and ovaries were removed about four months after the radiation treatments were completed.

The uterus was 9 cm in length and 6 by 4.5 cm at the fundus. The peritoneal covering was smooth and reddish tan. The entire endometrium and that portion of the myometrium immediately adjacent were replaced by a foul-smelling, shaggy, green membrane to which, in the left cornu, a putty-like mass of necrotic, darker green tissue was attached. This mass measured 2 by 1 by 1 cm. The myometrium varied in thickness from 1 to 1.5 cm. It was white and glistening in its outer half. Nearer the necrotic lining the myometrium was pale green. A darker green layer of myometrium, 3 mm wide, adjoined the dark green necrotic lining. The cervical canal was lined by a red, soft membrane, beneath which the pliable white tissues of the fibromuscular coat could be seen.

Several blocks were taken from various parts of the uterus and fixed in 4 per cent solution of formaldehyde or Bouin's fluid. Paraffin sections were stained with hematoxylin and eosin.

*Microscopic Examination*—The entire endometrium and endocervix were replaced by a layer of necrotic debris. In the body this was rather wide. It was composed of amorphous, poorly staining material in which masses of bacteria could be seen. The lower part of this layer was densely infiltrated by neutrophils, deep to which was a partially hyalinized zone of edematous myometrium, rather heavily infiltrated by neutrophils. This was not sharply demarcated from a wide zone of underlying edematous myometrium. The edema in areas extended almost to the serosa. It was accompanied by some neutrophilic infiltration. Near the serosa small clumps of lymphocytes, plasma cells and monocytes were seen.

In the hyaline myometrial zone there were definitely hyalinized, palely staining muscle fibers. The hyaline material in this zone was very pale. In this same zone, as well as in the necrotic zone above it, fibrinoid necrosis of small blood vessels had occurred. Infiltration of the swollen intima of some arteries by neutrophils and even complete necrosis of parts of the walls of arteries with thrombosis were seen, particularly where the necrotic surface layer extended deep enough to involve parts of the walls of larger arteries.

In the edematous myometrial zone there were a few giant endothelial cells and fibroblasts. Swollen endothelial cells appeared in small arteries and were often associated with intimal edema and deposits of fibrin or even neutrophilic infiltration.

On portions of the serosa a thin layer of fibrin and a few neutrophils could be seen.

Deep to the necrotic material and neutrophils lining the cervical canal lay a thin layer of hyalinized fibromuscular tissue heavily infiltrated by plasma cells and lymphocytes. Near the upper end of the cervical canal, the hyalinization of the fibromuscular coat extended deeper, and a hyaline clot appeared on the surface. Arterial changes were marked here, with hyaline intimal thickening, often massive, and thrombosis in evidence. Deep in the cervical wall here similar arterial changes could be seen.

*Summary*—The observations included acute gangrenous endometritis and endocervicitis with extension of the inflammatory process into the adjacent myometrium and cervical wall, acute diffuse metritis, marked near the necrotic lining, a completely necrotic polypoid mass in the left cornu, chronic cervicitis, mild focal acute perimetritis, mild acute perisalpingitis, atrophy of the ovaries, no evidence of carcinoma.

**CASE 6**—A patient aged 42 complained of pain in the back and of a greenish vaginal discharge which had been present for six months. The menstrual periods had always been irregular, but they had been particularly so during the six months preceding entry into the hospital. A diagnosis of adenocarcinoma of the endometrium (grade 2) was made on examination of material obtained by curettage. Radium therapy was begun. The patient had received a dose of only 1,800 milligram hours when her condition indicated that the treatment should be stopped. No roentgen radiation was used. About one month later the uterus and adnexa were removed.

The uterus measured 9 cm in length and 7 by 4.5 cm at the fundus. The peritoneal surface was smooth, glistening and pinkish yellow with dark red patches. The myometrium was 2 cm thick, yellowish white and fibrillary. The endometrium was thin (about 1 mm). At the level of the internal os a dark red, slightly elevated portion of the lining, 0.4 cm in diameter, was seen. The cervix was 3 cm in length and somewhat enlarged.

and boggy Beneath the membrane of the cervical canal there was one well demarcated nodule of shiny white whorled tissue, 0.5 cm in diameter

Several blocks were removed from the uterus and fixed in Bouin's fluid Sections were prepared as in the preceding case

*Summary of the Microscopic Examination*—Over large areas the endometrium and the innermost part of the myometrium were replaced by a layer of neutrophils, blood and debris The superficial portion of the adjacent myometrium was hyalinized No definite zone of edematous myometrium was seen The hyalinized zone was widest and the clot on the surface thickest at the internal os In the hyalinized zone, muscle fibers apparently undergoing gradual hyalinization were seen It was difficult, however, to decide whether they were being hyalinized or whether hyaline material was being deposited around the nuclear remnants of atrophic or necrotic muscle cells Where the surface layer of necrotic material and blood was thinnest there occurred considerable plasma cell infiltration with milder neutrophilic infiltration Hemosiderin was present too Where the hyalinized myometrial layer was very thin, no definite changes in the small arteries except fibrinoid necrosis could be seen Where this layer was thick, various other arterial changes were noted swelling of the endothelial cells, with fibrin or neutrophils or foam cell plaques in the intima The rest of the myometrium was normal No tumor was found in it

In areas the endometrium was still present A layer of fibrin or of fibrin and red cells with a few neutrophils overlay some parts of the surface epithelium and extended from the mouth of one gland to that of the one adjacent Similar material was found in the lumens of the glands The surface epithelial cells beneath the fibrin were swollen, glassy, or finely granular and deeply eosinophilic Some of the nuclei were large and vesicular or hyperchromatic, some were small and deeply basophilic (pyknotic) Beneath this altered epithelium in areas plasma cells and hemosiderin were seen, in other areas red cells, fibrin and dilated capillaries were present Edema was in evidence, but the connective tissue cells of the stroma appeared normal Here and there the surface epithelium was absent In such areas the fibrinous pseudomembrane on the surface was attached At such points blood clot partially hyalinized, was often seen Similar small clots, recent or old, were located beneath other parts of the surface epithelium and caused some protrusion of it into the lumen The epithelial cells overlying the clots were elongated and flattened and had elongated dark nuclei parallel to the surface

The epithelial cells in the glands were quite varied in appearance Many were large with large vesicular nuclei, round, oval, indented and lying parallel or at right angles to the lumen Some were so swollen that their free borders projected into the lumen Where the most superficial part of the endometrium, including parts of the glands, was replaced by fibrin or fibrin, red cells and neutrophils, the remnants of the glands were often lined by epithelial cells which differed greatly from one another (fig 6) Some were swollen and had large dense basophilic nuclei, others had swollen, pale vesicular nuclei, all had deeply eosinophilic cytoplasm The cell boundaries were poorly defined A few cells were multinucleated In one part of the endometrium, thicker than the rest, were masses of glands with large eosinophilic epithelial cells, often syncytial in appearance, with numerous round vesicular, somewhat enlarged nuclei These glands were con-

sidered to be carcinomatous In some of the glands, neutrophils, red cells, monocytes, fibrin or protein precipitates were seen

Near the internal os there was fairly deep hyalinization of the cervical tissues

*Summary*—The observations included acute hemorrhagic necrotizing endometritis with necrosis of the adjacent myometrium in areas, chronic metritis (inner portion of the myometrium near the necrotic lining), radiation-induced changes in the epithelial cells of the endometrium and its glands and in the residual carcinomatous glands, chronic endometritis with old and recent hemorrhages, radiation-induced arterial changes and a radionecrotic plaque in the uterine wall at the level of the internal os, chronic cervicitis with cystic dilatation of the glands, bilateral chronic salpingo-oophoritis and perisalpingo-oophoritis small tubo-ovarian abscess on the left side

#### SUMMARY OF THE OBSERVATIONS IN THE FOUR CASES IN WHICH THE UTERUS WAS SUBJECTED TO SPECIAL MICROSCOPIC EXAMINATION, RELATIONSHIP OF THE CHANGES TO RADIATION THERAPY

*Plaque at the Internal Os*—In each of the 4 cases a plaque of necrotic tissue was found at the level of the internal os of the uterus The plaque was definitely an effect of radiation The structure and the genesis of the plaque will be discussed in detail farther on

*Endometrium*—In each case the endometrium where present, was atrophic, often mildly edematous and at least focally infiltrated by chronic inflammatory cells, particularly near the plaque In 3 cases (1, 2 and 4) the menopause had occurred years before radiation therapy was instituted Hence atrophy of the endometrium might well have been the result of the cessation of ovarian function and cannot be definitely attributed directly to the irradiation of this tissue In case 3 radiation may have played a role either directly by action on the endometrium or indirectly through its effect on the ovaries These were definitely atrophic The chronic endometritis observed in these cases was diffuse, it was associated with old hemorrhage in case 3, localized in the region of the plaque in cases 1 and 2 and present in the only strip of endometrium found in the uterine body in case 4 It is probable that the chronic endometritis was an effect, direct or indirect, of irradiation of the uterus although the trauma resulting from the presence of radium applicator tubes in the uterus may have contributed The slight separation of the connective tissue cells seen in the more superficial layers of the endometrium in these cases and considered indicative of edema is not uncommonly seen in the nonirradiated atrophic endometrium Hence it cannot be proved to have

resulted from the irradiation. The diffuse necrosis of most of the endometrium in case 4 was probably the result of irradiation.

Squamous metaplasia of the columnar epithelium of the endometrium was observed in 1 case (3). No direct relation of this change to the radiation can be predicated. It is probable that the metaplasia was but a manifestation of the chronic endometritis, which was rather severe in this case.

*Myometrium*—One definite fact stands out—in none of the 4 cases did the profound atrophy occur which had been described as an effect of radiation. In 2 cases (1 and 3) the uterus was distinctly larger than normal. The presence of a few small foci of leiomyoma could not have been responsible for the large size. In case 1 the muscle bundles of the myometrium seemed to be of normal size or even somewhat larger than normal (fig 7). The only abnormality in the upper body were the arterial changes said to be characteristic of subinvolution of the uterus.<sup>2</sup>

In case 2 the muscle bundles in the inner half of the myometrium appeared somewhat atrophic, although the uterus as a whole was of about normal size. In this uterus, too, the arterial changes found in subinvolution of the uterus were in evidence.

In case 3, in which the uterus was large, there was a definite diffuse increase in connective tissue between the muscle bundles of the myometrium. The individual muscle fibers appeared enlarged (hypertrophy). It was difficult to determine accurately the size of the uterus in case 4 since it was received in several pieces. It was estimated to be of about normal size. The ectopic endometrium in the myometrium in this case (fig 8) might have been partly responsible for its size.

It must be concluded that at least in these cases no diffuse muscular atrophy or diffuse hyalinization with contraction was produced by radiation despite the fact that full "cancer doses" were employed. It is possible that the uterus in each instance was so large before being irradiated that even a notable shrinkage produced by radiation still left it larger than normal or about normal in size. This possibility does not seem very plausible but cannot be absolutely invalidated. The deviations from the normal seen in the myometrium in 2 cases—atrophy of the inner portion of the myometrium in one and fibrosis and mild hypertrophy in the other—are too diverse to be considered effects of radiation.

The clumps of lymphocytes noted along the small veins and lymphatics in the myometrium, evidence of chronic metritis, were most numerous

in those parts of the myometrium beneath or adjacent to the plaques. Their presence must have been the result of the inflammatory reaction in or near the plaques. Only a few of these clumps of lymphocytes were found in cases such as case 2, in which the inflammatory cellular response at the plaque was meager.

The focal acute metritis noted deep in the wall of the uterus in case 4 resulted from bacterial invasion of the plaque and so was an indirect result of irradiation.

Small foci of leiomyoma were found in the uterus in some of the cases. In most of them the neoplasm was only partly degenerated. Hyalinization, calcification and early cystic change were noted but were patchy in all. The muscle fibers in many seemed atrophic. These changes can be found in unirradiated leiomyoma and hence cannot be proved to have been produced by radiation. It is noteworthy that ectopic endometrium was present in the myometrium in 1 case in which the uterus was removed twelve years after the menopause. The ectopic endometrium showed the same degree of atrophy as the lining endometrium. As has been noted already this atrophy could not be proved to be due to radiation.

Most of the changes in the arteries in the myometrium near the plaque can be considered effects of radiation and will be considered in the discussion of the structure and the genesis of the plaques later in this paper. In the middle layer of the myometrium in 2 cases (1 and 2) definite swelling of elastic tissue, the so-called vitreous change, was seen in the walls of arteries. Occasionally apparently new small arteries were encased in wide expanses of altered degenerated elastic tissue, supposedly the remains of older larger arteries. These changes have been attributed to faulty involution of the uterus.<sup>2</sup> They are not radiation changes. The relation to radiation of hyalinization of small arteries in the depths of the endometrium or in the adjacent myometrium is more difficult to determine. In the 2 cases just mentioned (1 and 2), particularly in the first, many of these small arteries were hyalinized, but the endometrium was less damaged by radiation than in cases 3 and 4, and the larger arteries showed the changes accompanying subinvolution of the uterus. In case 3 hyalinization of similar small arteries was widespread, and the endometrium showed more radiation damage, but the arterial changes of uterine subinvolution were not present in the larger arteries. In case 4 the damage of the endometrium due to radiation was gravest; the arterial changes of subinvolution were not in

evidence in the large arteries of the middle layer and there were but few hyalinized arterioles near the endometrium. These observations seem to indicate that the hyalinization of the small arteries of the inner myometrium and the deeper portions of the endometrium may be a manifestation of uterine subinvolution or of radiation-induced change or possibly of some other condition and hence not a specific radiation change.

The peritoneum covering the uterus revealed no pathologic change directly attributable to radiation. The fibrous adhesions, the lymphocytic infiltration and the foreign body giant cell reaction to refractile foreign material in and on the serosa in case 1 could be the result of the old pelvic operation.

The marked chronic cervicitis noted particularly in cases 1 and 3 was probably indirectly the result of irradiation. The small ulcerated area on the vaginal aspect of the cervix in case 1 overlay an area of hyalinization of the fibromuscular coat in which radiation-induced vascular changes were present. There is little doubt that the changes in this part of the cervix were due to radiation.

The plasma cell infiltration deep in the wall of the cervix, particularly beneath the plaques, certainly was related, indirectly at least, to necrosis produced by radiation.

In short, the only uterine changes in the 4 cases which can be definitely considered effects of radiation are the plaque, the changes in the adjacent myometrium and the wall of the cervix, the arterial changes in the plaque and in the adjacent tissues, and the one area of extensive hyalinization of the cervix noted in case 1. The acute gangrenous endometritis and endocervicitis noted in case 4 and the chronic endometritis and cervicitis noted in the other cases are probably also the result of the irradiation though the modifying effects of trauma (introduction and removal of applicator tubes), hemorrhage and infection mask the more primary effects of radiation.

The most important effect of radiation on the uterus in these cases has not yet been discussed—the eradication of definite adenocarcinoma of the endometrium demonstrated histologically before the therapy was begun. In each case a plaque of necrotic tissue was found at the level of the internal os. Only in case 2 was a second necrotic mass encountered. In this case the mass was polypoid and so necrotic that no vestiges, even wraithlike, of former structures, carcinomatous or not, could be identified. In no case were scars found in the myometrium. Only in case 2 were small calcified bodies encountered, possibly calcified cancer cells. In this case it

is possible that the cancer had been present in the polypoid mass. In the other cases no probable site of the cancer was identified. At first it was thought that the necrotic plaques at the internal os were necrotic tumors, but later they were considered to be areas of radionecrosis. Of course the cancer might have been located at the level of the internal os and destroyed along with the normal structures at this site by the caustic action of the rays. It is also possible that the carcinoma was superficial, removed by curettage or completely destroyed by radiation. Endometrial reparative processes might then have hidden the site. However, in this paper photomicrographs of the cancer (figs 1 and 2) reveal a histologic character different from that usually seen in the type strictly limited to the mucosa. From the histologic observations one would anticipate invasion of the myometrium. Yet no evidence of such infiltration was found in these 4 cases.

Finally, with the method of examination used in our cases it is unlikely that residual carcinoma was missed. To our knowledge, in previous reports on the changes in uteri excised after radiation therapy for carcinoma of the corpus uteri it was merely stated that there was no microscopic evidence of residual carcinoma (Healy and Brown<sup>4</sup>, Martin<sup>5</sup>) or that no special search had been made (Donovan and Warren<sup>6</sup>), or else microscopic observations, apparently on routine sections, were given without a statement of the details of the method of examination used (Aineson<sup>7</sup>, Corscaden<sup>8</sup>).

No definite indication of the mode of destruction of the cancer is afforded by the observations in the cases reported in this paper.

#### IMPORTANT FEATURES OF THE RADIONECROTIC PLAQUES AT THE LEVEL OF THE INTERNAL OS

*Gross Features*—In all the cases the plaque was an irregular mass of partly soft and partly firm material. The size was variable from case to case, and the surface was elevated above the adjacent portions of the endometrium, from which in some cases the plaque was well demarcated (case 3), in others, it was not (case 4). Its no limits were apparent the uterus was usually

4 Healy, W. P., and Brown, R. L. *Am J Obst & Gynec* **38** 1, 1939.

5 Martin, C. L. *South M J* **33** 135, 1940.

6 Donovan, M. S., and Warren, S. *Surg, Gynec & Obst* **74** 1106, 1942.

7 Aineson, A. N. *Am J Roentgenol* **36** 461, 1936.

8 Corscaden, J. A. *J A M A* **126** 1134, 1944.

lined by necrotic material, and the plaque was but a particularly thick portion of the necrotic lining

For the most part the color of the material in the plaque was some shade of brown or green. In case 6, in which the plaque could not have been present longer than one month, the earliest stage observed, the color was dark red. The hemorrhage which played a part in the production of the plaque and in the determination of its structure in all cases was probably responsible for the shade of red in the earlier stage and of brown in the later one. Microscopically, at the latter stage the plaque was composed, at least in part, of old hyalinized clot and hence was brownish. The green tints were probably the result of bacterial invasion and activity, since they were seen in the cases in which bacterial masses were found either in the plaque (case 4) or in the necrotic uterine lining (case 5).

The plaque not only projected into the uterine cavity (even as much as 6 mm) but also extended into the adjacent myometrium (but no deeper than 5 mm). The base of the plaque was usually well demarcated from the adjacent myometrium. In fact, in some of the cases the structure was easily dislodged.

In the cases in which the plaque was smaller (6 mm in diameter) it was located in its entirety at the level of the internal os. In the cases in which it was larger (2.5 and 3.3 cm in length) it extended up from the internal os into the body rather than down along the cervical canal and usually encircled the lumen.

*Microscopic Structure*—Typically the plaque (fig 3) was an infarct-like surface zone of complete necrosis. Beneath it typically lay a zone of partial hyalinization and edema and finally between this hyaline-edematous zone and the normal tissues a zone of edema and possibly of atrophy. The typical structure was seen best on the uterine body side of the internal os. Here the three zones could be readily defined and designated: the zone of complete necrosis, the zone of hyaline-edematous myometrium and the zone of edematous myometrium. The altered myometrium adjoining the necrotic surface zone was not merely a zone of reaction to the presence of necrotic tissue; it too showed the direct effect of the radiation.

In the typical necrotic surface zone there was a finely granular or faintly fibrillary pale material (case 1) or a more compact pale hyaline-like material (case 2). In it outlines of necrotic blood vessels and even faint tracings of muscle bundles could be identified. The exact size of

the necrotic blood vessels in this zone and the presence or the absence of necrotic muscle bundles depended on the thickness of the necrotic zone, i.e., the depth of involvement of the myometrium or of the fibromuscular coat of the cervix. The endometrium was always destroyed. In some cases necrobiotic endometrial glands persisted near the free surface. Epithelium continuous with that on the surface of the adjacent endometrium or endocervix extended for variable distances up over the margins of the plaque in the typical case.

The hyaline-edematous zone and the edematous zone were often relatively thin (0.5 to 1 mm). In the former, typically only nuclear remains of muscle cells persisted, with deposits of palely staining hyaline material around small blood vessels or even around the necrotic muscle cells themselves (fig 4). In the latter case naked pyknotic nuclei set in small empty spaces were separated from adjacent nuclei by thin bands of hyaline material.

In the edematous zone the muscle fibers were separated by spaces and appeared thinner than normal. As the hyaline zone was approached the nuclei of the muscle cells became more pyknotic, and the cytoplasm shrank and finally disappeared. The hyaline-edematous and edematous zones were not always sharply separated from each other. The hyaline-edematous zone, however, was usually well demarcated from the zone of complete necrosis. In case 2 two distinct zones were not found. In large areas practically normal myometrium adjoined the plaque.

In a typical case (case 1) over fairly large areas no cellular infiltration was noted in any zone with the exception of the completely necrotic zone, and here the infiltration was mild. Even here many structures which at first sight appeared to be neutrophils proved to be pyknotic nuclei of necrotic muscle cells. Over fairly wide areas there was also no cellular reaction in the more normal tissues bordering on the plaque. In areas, however, there was diffuse or focal cellular infiltration, at times massive, particularly in the hyaline-edematous or the edematous zone but sometimes in the lower part of the necrotic zone or in the normal tissues near the plaque. The infiltration was best seen where hemorrhage or bacterial invasion had caused deviations from the typical structure of the plaque and of the adjacent zones of altered tissue.

In general, on the cervical side of the internal os the distinction between the hyaline-edematous zone and the zone of edema was not well defined. Often a hyaline zone much more compact than the hyaline-edematous myometrial zone was in



contact with the zone of complete necrosis on the surface

In general there was no granulation tissue zone in contact with the necrotic tissue. In case 3, however, the complete disappearance of muscle elements and the persistence of connective tissue and blood vessels gave a definite impression of the presence of granulation tissue (fig 4).

In the typical case bizarre giant fibroblasts or endothelial cells were present only in a few areas.

#### *Modifications in the Structure of the Necrotic Plaques Produced by Hemorrhage and Infection*

—The typical plaque was seen in 2 cases (1 and 2). It was modified in all the other cases and even in places in cases 1 and 2. The two predominant causes of modification were hemorrhage and bacterial invasion.

Masses of red cells were present in areas even deep in the typical plaque. It was not uncommon to find masses of fused red cells or of hyalinized dark red clot over all of the surface of the infarct-like portion of the plaque or on parts of it only. If the hemorrhage was more massive, the infarct-like zone might be completely replaced by altered hyalinized clot or at least by necrotic tissues so saturated with altered blood that they appeared to be hyalinized clot. This occurred in case 3, in which hyalinized clot rested directly on the hyaline-edematous myometrial zone (fig 4).

Where clot rested directly on the hyaline zone there was a definite tendency for the plaque to be infiltrated by plasma cells, lymphocytes and monocytes in particular. Fewer neutrophils and eosinophils were seen. Red cells and hemosiderin-laden macrophages, as well as extracellular hemosiderin deposits of varying size, were often present.

Bacterial invasion was the other common cause of modification of the structure of the plaque. Where it was focal a nice contrast with the adjacent more typical plaque was afforded (cases 3 and 4). The entire invaded portion stained poorly and appeared partially autolyzed. Myriads of bacteria were present. Deep to this amorphous zone was a dense layer of neutrophils resting on a wide hyaline myometrial zone or a hyaline-edematous zone, rather heavily infiltrated by neutrophils. In this hyaline zone which stained faintly, definite hyalinization of muscle fibers, sometimes with swelling, occurred. Hyalinized muscle cells were rarely seen except where bacterial invasion altered the plaque. The nuclei of these muscle cells faded gradually

*Arterial Changes Associated with the Plaque*—A variety of arterial changes were seen in and adjacent to the plaque. On the uterine body side the changes were most often in the hyaline-edematous or the edematous myometrial zone next most commonly in the depths of the zone of necrosis and least often in the normal myometrium. On the cervical side the vascular changes were not only adjacent to the plaque but also at considerable distances away deep in the fibromuscular coat (fig 5).

The following arterial changes were observed: swelling of endothelial cells, with or without the subendothelial accumulation of fluid fibrin, red cells, and a few inflammatory cells, particularly lymphocytes, monocytes and neutrophils, absence of endothelium, foam cell intimal plaques<sup>3</sup>, hyaline intimal thickening, even occlusive hyaline necrosis of various portions of the arterial wall with or without dilatation, neutrophilic infiltration, hemorrhage and deposition of fibrin, more rarely fibroblastic intimal thickening and thrombosis with partial organization. Occasionally hemosiderin deposits in hyalin-thickened intima were seen. Some of these arterial changes are depicted in figure 5; others are illustrated in a previous article<sup>3</sup>. All of these in our opinion were due to radiation. Acute diffuse arteritis with necrosis, edema of the entire arterial wall with or without neutrophilic infiltration and fibrinoid necrosis were observed most commonly near areas of bacterial invasion. Undoubtedly this invasion played some part in their production, particularly where completely necrotic portions of the walls of arteries with massive neutrophilic infiltration were in contact with necrotic material containing masses of neutrophils and bacteria.

Hyalinization in varying degrees of severity was most common in arteries less than 300 microns in external diameter and was more common on the cervical side of the plaque than on the uterine body side. Hyalinization of arteries was present on the body side particularly when the plaque did not penetrate deeply into the myometrium (case 2).

#### THE GENESIS OF THE PLAQUE

It is evident from the description of the properties of the plaque that it is essentially a zone of coagulation necrosis. The coagulation necrosis is identical with that found in many bland infarcts elsewhere in the body. The admixture of variable amounts of blood with the necrotic tissue is also usual in infarcts elsewhere. Swelling is a feature of such infarcts. It was found



in the uterine plaque, which in most of the cases projected somewhat into the uterine cavity. The swelling is probably due in both instances to the outpouring of protein-containing fluid and red cells into the necrotic area and subsequent coagulation. Like true infarcts, the typical radionecrotic plaque in the uterus is well demarcated from the adjacent viable tissues, in the latter in both instances marked changes in the walls and lumens of arteries are usually seen. When the uterine plaque becomes secondarily infected the changes simulate those of septic infarction.

There are however, some apparent differences, notably, either the lack of a cellular inflammatory reaction in the areas of radionecrosis or in the surrounding viable tissues or at the most mild infiltration, the lack of granulation tissue in the typical case, and finally the long persistence of the radionecrotic tissues even when located on a surface. In addition, the diffuse hyalinization of the tissues bordering on the areas of radionecrosis, the obliteration of the capillary bed in the surrounding tissues and some features of the local arterial changes are not commonly observed in tissues adjacent to ordinary bland infarcts. However, if a comparison is made with old bland infarcts rather than with recent ones the differences are not so striking. Around the periphery of an old splenic infarct, for example, it is not unusual to find at least a narrow band of partially hyalinized connective tissue with little cellular infiltration. However, diffuse hyalinization extending some distance from infarcts is not usual. Since in most of the cases described in this paper the uterine plaque was two and a half or more months old, a comparison with the older bland infarcts is probably appropriate.

The youngest plaque encountered in our material (case 6) was somewhat less than one month old. Numerous neutrophils were found in it. These may have been due to the infection present. In the endometrium adjacent to the plaque in this case small areas of necrosis limited to the most superficial layer of the endometrium were present (fig. 6). In the adjacent endometrial stroma edema, deposition of fibrin, fibrinoid necrosis of capillaries and of arterioles, dilatation of capillaries and small hemorrhages occurred. In the same region small clots, still retaining hemoglobin (orange-colored) or hyalinized (dehemoglobinized), were found just beneath the surface epithelium, portions of which were pushed outward into the uterine cavity by these deposits. The only definite evidence of necrosis here was the necrobiotic changes in the overlying surface epithelium. Perhaps such

areas represent the early stage of plaque formation. The later desquamation of the epithelium with deposition of fibrin on the surface and among the subjacent connective tissue cells would simulate small infarcts.

Since the areas of radionecrosis are essentially areas of coagulation necrosis, they must result from the necrosis, in a relatively short interval of time, of all cells, epithelial, endothelial, fibroblastic and muscle, sufficiently close to the source of radiation, or from primary involvement of the blood vessels with occlusion and secondary infarction, or possibly from some change in the interstitial tissue or its fluids which interferes with the nutrition of the cells in the area. In favor of infarction is the observation that the seriously damaged portions of arteries are concentrated in the tissues close to the areas of necrosis. On the body side of the internal os in most of the cases these arterial changes were largely but not entirely confined to a layer of myometrium immediately adjacent to the dead tissue. All arteries passing through this fairly thin myometrial layer showed equivalent changes. Since these arteries ran parallel to one another, perpendicular to the lining of the uterine cavity, those who consider the areas of radionecrosis to be true infarcts must, in view of the close proximity of the occluded blood vessels to the area of necrosis, postulate coalescence of multiple small infarcts to explain the large plaque found in some of the cases. The problem of the exact mechanism of the production of areas of radionecrosis must remain unsettled until the relative importance of the various factors possibly concerned can be assessed. These include direct damage of individual cells, hyalinization of connective and of other tissues, vascular damage and possibly changes in the interstitial fluids. Even the origin of the various forms of hyalin encountered in the areas of radionecrosis and in the adjacent tissues is not clear. There are several possible sources: hyalinized blood clot, precipitated or gelled protein, old fibrin, hyalinized collagen and hyalinized cells.

The location of the plaque in the uterus and the relative roles of roentgen radiation and radium in its production remain to be discussed. In 1 case in which a well defined plaque was found, radium alone was used. It is reasonable to conclude that the radiations from radium produced the plaque in all the cases. The radium was inserted into the arms and the stem of a Y-shaped applicator so constructed that, once inserted, the arms could be spread apart to rest against the lateral wall of the uterine cavity.

above the internal os. The stem remained in the cervical canal. It is highly probable that that part of the uterine wall at the level of the internal os was most closely applied to the source of radiation and that the effects of the latter were most intense there. The portions of the wall just above the internal os would receive a dose somewhat less but still quite intense. The shape of the mechanical applicator and the distribution of the radium in the arms and stem thus account for the location of the plaque. It is interesting that in a thick-walled organ such as the uterus a dose of radium sufficient to cause complete necrosis can be applied with little danger of serious damage, such as perforation. The main hazard is infection, which was present in some of the cases reported here. However all of the patients whose cases are reported here are still alive two years or more after irradiation of the uterus and hysterectomy.

#### HISTORICAL SKETCH

Necrosis, slight or extensive, diffuse or focal of the walls of the uterus subjected to radiation has been noted by numerous investigators. In the older literature Haendly<sup>9</sup> and Adler,<sup>10</sup> particularly the former, listed a variety of changes or gave detailed descriptions. Adler mentioned necrotizing inflammation of the uterus, diphtheritic endometritis, and "endometritis and metritis purulenta." He dealt mainly with cervical carcinoma. Haendly<sup>9b</sup> stated that there was slight necrosis of the superficial portion of the wall of the uterus in all cases in which the uterus was treated with radium or mesothorium but that it was not important. As more damaging effects of radiation he mentioned necrosis of the entire cervix and corpus in 1 case, gangrene of the uterus in 1, necrosis of the entire fundal portion of the uterus with sepsis, necrosis of the cervix and a portion of the corpus, and gangrene of the anterior or the posterior wall of the uterus in other cases. In 1 case "endometritis gangrenosa" and in 4 others "endometritis nekroticans" or "endometritis nekrotica septica" were diagnosed. He also laid great stress on hyaline degeneration, with or without necrosis, of the connective tissues and of smooth muscle in the muscular myometrium as well as in the walls of blood vessels. Many of Haen-

dly's cases in which the patient came to grief were cases in which cancer of the cervix was treated with mesothorium or mesothorium and radium. The role of infection and of improper filtration or dosage in the production of the necrosis in his cases, perhaps, was not sufficiently well evaluated.

More recently Meyer<sup>11</sup> mentioned several layers in irradiated carcinoma of the corpus: a superficial zone of necrosis, a subjacent zone of necrobiosis and a deep infiltration or granulation tissue zone. Todd<sup>12</sup> noted radionecrosis of the whole lining of the uterus in a case in his series. Arneson,<sup>7</sup> in a case in his series, found the uterine cavity lined with a layer of hyalinized tissue 3 to 4 mm thick, with completely degenerated and unidentifiable cells in the deeper portions. Deep to this layer of necrosis the muscle cells showed hyalinization to a depth of several millimeters. Occasional thin-walled blood vessels were found along the line of demarcation between the superficial layer of necrosis and the underlying myometrium. He interpreted the presence of these blood vessels as a manifestation of an attempt at repair.

Localized areas of necrosis comparable to that described as a radionecrotic plaque in this paper have also been remarked on. Letulle<sup>13</sup> stated that radium causes deep cauterized lesions in uterine cancers. The scar in his case, corresponding to our radionecrotic plaque, was dry and hard and was characterized by long persistence, by long adherence to the normal tissues at the periphery, by prolonged inhibition of the usual inflammatory reaction and by fibrinoid necrosis of vessels which he felt was a most important sign of the causticity of radium.

Clark and Norris,<sup>14</sup> in summarizing their findings in numerous uteri subjected to radium reiterated that there is no question but that radium exerts local action on the inner surface of the uterus, characterized at times by localized sloughing or necrotic areas involving the proximal layers of the myometrium, especially in those cases in which a moderately thorough curettage preceded the irradiation. In their cases the sloughs were at times as large as 5 cm in length and 1 to 1.5 cm in width but usually were smaller, they were located on either the anterior

9 Haendly, P. (a) *Arch f Gynak* **109** 409, 1918, (b) *Strahlentherapie* **12** 1, 1921.

10 Adler, L. *Die Radiumbehandlung maligner Tumoren in der Gynakologie*, in Falta, W., Gauss, C. J., Meyer, H., und Werner, R. *Sonderbande zur Strahlentherapie*, Berlin, Urban & Schwarzenberg, 1919, vol. 4.

11 Meyer, R. *Die pathologische Anatomie der Gebärmutter*, in Henke, F., and Lubarsch, O. *Handbuch der speziellen pathologischen Anatomie und Histologie*, Berlin, Julius Springer, 1930, vol. 7, pt. 1, p. 477.

12 Todd, T. F. *Lancet* **2** 821, 1938.

13 Letulle, M. *Presse med* **30** 121, 1922.

14 Clark, J. G., and Norris, C. C. *Radium in Gynecology*, Philadelphia, J. B. Lippincott Company, 1927, pp. 129-131.

or the posterior wall usually the latter. The area of necrosis healed slowly, often persisting for weeks or months. Months after treatment macroscopic or histologic evidence of the action of radium might be absent. While localized fibrosis of the myometrium at the site of previous irradiation might be present, no massive cicatrices were encountered. Changes in blood vessels were quite localized to the site irradiated. These were generally moderate in degree and included thrombosis of the smaller uterine blood vessels.

Pullinger<sup>15</sup> classified such localized areas of radionecrosis in the uterus as infarcts and stated that they were examples of infarction at a surface. She claimed also to have seen rounded infarcts in the smooth muscle of the uterus. There was no detailed description of the necrotic patches. MacKee<sup>16</sup> considered similar areas of necrosis in the skin in third degree cutaneous roentgen ray reactions examples of dry gangrene. Corscaden<sup>8</sup> commented on the apparently localized effect of radium in the uterus. The sloughs and areas of cellular degeneration in his cases were limited in area and depth, giving him the impression that they corresponded to the location of the radium applicators. In a case in the series reported by Arneson and Hauptman<sup>17</sup> the necrosis and ulceration involved the cervical canal and the lower portion of the uterine cavity.

Vascular changes associated with areas of necrosis in the irradiated uterus have been stressed by Letulle,<sup>13</sup> Haendly<sup>10</sup> and Pullinger<sup>15</sup>. In some of the other reports previously cited, there is mention of vascular changes but no detailed report. Dyroff<sup>18</sup> noted vascular changes. Haendly<sup>10</sup> observed capillary dilatation and stasis and changes in the walls of larger vessels. In the latter the end result of the changes was in his opinion a hyaline dead mantle, sometimes calcified, surrounding the thrombosed or obliterated lumen, even though at times the intima, at times the media or the adventitia, was most affected.

There has been considerable speculation on the genesis of localized areas of radionecrosis, whether in the uterus or elsewhere. Letulle<sup>13</sup> attributed the necrosis in his case to the causticity of radium without further explanation, Lacassagne,<sup>19</sup> in commenting on Letulle's case, attributed the necrosis to the action of  $\beta$  rays and

to the soft rays ( $\gamma$  *mol*) of radium. He stated that the use of hard rays ( $\gamma$  *dur*) would have prevented frank necrosis of normal tissues. Dobrovolskaia-Zavadskaia,<sup>20</sup> in her experimental work, described two zones concentrically placed around a source of radiation (radium and radon): an inner zone of necrosis and an outer zone of atrophy. The former she attributed to the action of soft rays ( $\gamma$  *mol*), the latter, to hard rays ( $\gamma$  *dur*). She believed that the centering of the zone of necrosis on the source of radiation ruled out vascular changes as the prime factor in the production of necrosis since the areas of necrosis did not correspond to the anatomic distribution of the local blood supply. Pullinger<sup>15</sup> considered the necrotic areas to be true infarcts and frankly attributed them to the vascular changes noted. She claimed that the following series of events occurred: hyperemia with stretching of the walls of capillaries and venules, resulting endothelial damage with exudation of serum and formation of small platelet thrombi, complete thrombosis of capillaries and venules and even of larger vessels, leading to necrobiosis and anemic necrosis. In the uterus the thrombosis was followed by coagulation of the mucous membrane with subsequent sloughing. Deeper involvement of vessels led to gradual extension backward into the uterine wall. Healing then occurred by formation of granulation tissue and replacement of the necrotic areas by fibrous tissue unless at the very beginning larger vessels were thrombosed. Then areas of quiet necrosis would persist. Her conclusions were challenged by Desjardins<sup>21</sup> on several grounds. Conclusions based on the use of full or tolerance doses such as those used in the material examined by Pullinger, in his opinion could not furnish a satisfactory answer to the problem of the mechanism of cell death in irradiated tissues. The vascular changes could well be secondary to the direct destruction of cells and tissues by radiation rather than primary radiation changes resulting in cell death. If the vascular changes were primary, one must conclude that at least some of the cells constituting the tissues in the blood vessel walls were injured more readily by radiation than cells outside the vessels. According to his statement, doses affecting certain other cells do not affect endothelial cells; hence one could postulate that the changes in the latter were secondary to the destruction of the former cells. He mentioned in particular such radio-

15 Pullinger, B. D. J. Path. & Bact. **35** 527, 1932.

16 MacKee, G. M. Roentgen Ray Reactions and Injuries, in Pohle, E. A. Clinical Roentgen Therapy, Philadelphia, Lea & Febiger, 1938, p. 743.

17 Arneson, A. N., and Hauptman, H. J. A. M. A. **116** 29, 1941.

18 Dyroff, R. Arch. f. Gynak. **136** 141, 1929.

19 Lacassagne, A. Presse med. **30** 323, 1922.

20 Dobrovolskaia-Zavadskaia, N. Lyon chir. **21** 397, 1924.

21 Desjardins, A. U. Am. J. Roentgenol. **28** 398, 1932.

sensitive cells as those of the seminal epithelium. That the exact genesis remains unsettled must be concluded from the foregoing discussion.

With regard to generalized atrophy of the myometrium, Haendly<sup>9b</sup> maintained that radiation caused two types of atrophy of smooth muscle, one characterized by deposition of hyalin and destruction of muscle nuclei, the other, by disappearance of the cytoplasm of the muscle cells with preservation of the nuclei. The latter appeared closely set. He claimed that the first type of atrophy was the more common in irradiated uteri and that because of it the uterus became a hard and shrunken senile organ. He was of the opinion that cessation of ovarian activity resulting from the use of radiation played little part, the shrinking of the uterus was due directly to radiation. In the cases now described no generalized atrophy of smooth muscle of either of these types was noted, despite the large doses of radiation used. The method of treatment—the total dose, the individual doses, the spacing of the doses and the filters used in our cases—might explain the difference in observed effects.

Finally, an interesting observation by Todd<sup>12</sup> should be mentioned. In 3 cases, in which the uterus was removed nine to nineteen months after a single dose of about 2,400 milligram hours, he found the normal endometrium replaced by a smooth shining membrane with telangiectatic zones and ruptured varices, the latter in 1 case situated just above the internal os. No information was given with regard to the microscopic observations.

#### SUMMARY

A thorough gross and microscopic study was made of 4 uteri excised after eradication of carcinoma of the endometrium by large doses of radium (about 6,000 milligram hours) and of roentgen radiation (about 4000 r in the mid-pelvis). Two other irradiated uteri were taken into consideration, although only routine sections of these were available. A carcinoma of the endometrium in one of these was destroyed by radiation.

The original site of the carcinoma in 5 of the 6 uteri could not be determined.

A localized plaque-like area of radionecrosis, essentially an area of coagulation necrosis, was found in the lining at or near the level of the internal os in 5 of the uteri. Changes were produced in the plaque by hemorrhage and infection. There is a question as to whether or not plaques of the type observed are true infarcts.

In the myometrial tissues adjacent to the uterine plaque two zones showing the effects of radiation were found: a superficial zone of hyalinization and edema with necrobiotic changes and a deeper zone of edema with atrophic changes. In the cervix a single zone of hyalinization was the usual finding. Vascular changes were encountered in these zones but were not confined to them.

Other observations included some degree of chronic endometritis, chronic cervicitis and endometrial atrophy, chronic metritis, mild and more or less focal, and other nonspecific lesions, including squamous metaplasia of the endometrial epithelium in 1 case.

# HEALING OF WOUNDS IN THE SKIN OF MICE PAINTED WITH 20-METHYLCHOLANTHRENE

MARTIN SILBERBERG, M D, AND RUTH SILBERBERG, M D

ST LOUIS

Benzpyrene applied to the epidermis of mice previous to the making of a wound in the skin stimulated the growth processes in the original as well as in the regenerating epithelium<sup>1</sup>. However, after prolonged painting with this substance, the ameboid movement of the proliferating epithelium into the defect was delayed or not increased at a rate commensurate with the intensification of the growth process, depending on duration of treatment. In order to determine whether this dissociation of growth and migration of the cells was due specifically to the action of benzpyrene or could be produced by other carcinogenic agents also, we have investigated the effect of 20-methylcholanthrene on the course of repair of the epidermis of mice treated with this carcinogenic substance previous to the making of the wound.

## MATERIAL AND METHODS

Sixty-four white Swiss mice, 2 to 3 months old at the beginning of the experiment, were used, being equally distributed as to litter mates and sexes whenever possible. The arrangement of the experiments was the same as in the previous investigation on the influence of benzpyrene with the exception that methylcholanthrene was substituted for the benzpyrene and that the carcinogen was applied by one stroke with a camel's hair brush (no. 6) instead of with a dropper.<sup>2</sup>

The animals were divided into three series. In the first series (16 mice) all the animals remained untreated as controls. In the second series (16 mice) the skin was painted with benzene three times weekly for two weeks, one month, two months or three months. In the third series (32 mice) the skin was painted with 20-methylcholanthrene dissolved in benzene in the concentration of 0.3 per cent. The carcinogen was applied for two weeks, one month, two months or three months.

At the end of the period of treatment the hair of those mice on which hair had grown in the interval was clipped a second time. An area of skin was marked

with a round stencil measuring 3 mm in diameter. This marked area was carefully excised with a pair of curved scissors, and regeneration was allowed to take place for three, five, eight or ten days. At the end of each experimental period, all mice were killed at the same time of the day, between 10 and 11 a. m., in order to avoid variations of the mitotic count due to the diurnal mitotic rhythm. A piece of skin including the wound was taken out and stretched on filter paper, the specimens were then fixed in 10 per cent formaldehyde solution and embedded in paraffin, the sections, 5 microns thick, were stained with hematoxylin and eosin.

The histologic observations are presented in tables 1 to 3. The ratio of basal to spinous cells is shown in column 4. The thickness of the epithelial layers was determined by counting the number of cell rows, and the size of the cells was considered in the various areas. The state of the original epithelium at a distance from the line of excision is seen in column 5, that of the epithelium at the margin of the wound, in column 6, and, lastly, that of the regenerating epithelial tongues advancing from the margin toward the center of the defect, in column 7. The length of the tongues is found in column 8. The mitotic count for the original epithelium (column 9) and that for the new epithelium (column 10) are given in multiples of the normal, the latter being in this strain 12 mitoses in 10,000 basal cells. As to further details of the method we refer to our former papers.

## OBSERVATIONS

*First Series* (control mice, untreated).—There was no difference in the progress of wound healing in the groups observed for two weeks, one, two or three months previous to excision. The following description is therefore concerned only with the different stages of regeneration and disregards the preceding period of observation.

(a) *Old Epithelium*. At a distance from the line of excision the epidermis at all stages was composed of two layers of epithelial cells, the upper consisting of spinous cells, the deeper of ellipsoid basal cells. The ratio of basal to spinous cells ranged between 2:1 and 2.5:1.

Near the line of excision the epithelium was thickened, three and five days after the making of the wound the epithelium was composed of four to six rows of cells instead of two as is normal. The spinous cells formed a thick layer of keratin. The basal cells were from 20 to 30 per cent larger than usual, cylindrical in shape and oriented in a direction perpendicular to the surface. The nuclei were likewise enlarged, the structure of the nucleoli had become indefinite. Mitotic proliferation of the basal cells was intensified, showing a maximum of eight times the normal five days after operation (table 1, column 9). From eight to ten days after excision the number of cell rows was four to seven, the basal cells were now less hypertrophic, and after ten days a return to the resting state had occurred in

This investigation was aided by the David May-Florence G. May Fund.

These experiments were carried out in the Department of Pathology, New York University College of Medicine.

From the Laboratory of the Jewish Hospital and the Snodgrass Laboratory of Pathology of the St. Louis City Hospital.

<sup>1</sup> Silberberg, M., and Silberberg, R. *Am. J. Path.* 20: 809, 1944.

<sup>2</sup> Silberberg, M., and Silberberg, R. *Arch. Path.* 38: 215, 1944.

some instances Likewise, the mitotic count began to drop, but even after ten days it still remained elevated, the mean values varying from three and a half to one and a half times the normal after eight and ten days, respectively

(b) *New Epithelium* Enlarged epithelial cells migrated into the defect, growing out in a tongue-like fashion under the blood crust covering the defect The advancing epithelial cells were spindle shaped, more so the closer they were to the tip of the tongue The length of the tongues increased gradually from a mean of 0.29 mm after three days to 0.87 mm after ten days of observation (table 1, column 8) Only in 1 of 4 cases

*Second Series Group 1* (benzene-treated mice, benzene applied for two weeks and for one month previous to the making of the wound) —(a) *Old Epithelium* The epidermis at a distance from the wound consisted of two to three rows of epithelial cells, the ratio of basal to spinous cells having shifted in favor of the latter (table 2, columns 4 and 5) In the subcutaneous tissue there were scattered polymorphonuclear and mononuclear leukocytes, and here and there the epithelial cells showed a karyorrhexis, karyolysis or pyknosis of the nuclei At the line of excision the epithelium was markedly thickened (four to nine cell rows), the maximum thickness of eight to nine rows being reached

TABLE 1—*Untreated Animals*

Duration of Treatment	Days After Excision	Animals	Ratio of Basal to Spinous Cells	Rows of Cells in			Length of Tongue, Mm	Mitosis in Multiples of the Normal Mean, Maximum and Minimum Values	
				Distant Epithelium	Insertion of Tongue	Tongue		Original Epithelium	New Epithelium
2 weeks	3	323	2:1	2	4-5	2-3	0.31	5 max 6½ min 4	½ max 1 min 0
	5	285	2:3:1	2	5	3-4	0.51	5½ max 6 min 5	6 max 6½ min 5½
	8	230	2:3:1	2	5-6	3-4	0.62	3½ max 5 min 2½	4½ max 5 min 3
	10	243	2:1	2	4	4	1.01	1½ max 2 min 1	3 max 4½ min 2
1 month	3	247	2:1	2	4-5	2	0.29	2½ max 3 min 2	0
	5	251	2:5:1	2	5-6	2-3	0.52	4 max 6 min 3	4 max 6 min 3
	8	255	2:1	2	4-5	4-5	0.66	2 max 3 min 1	2½ max 3½ min 1½
	10	259	2:5:1	2	4-5	2-3	Closing	2 max 2 min 2	3½ max 4 min 3
2 months	3	263	2:5:1	2	4-5	2-3	0.27	5½ max 7 min 4	¼ max ½ min 0
	5	267	2:2:1	2	5-6	2-3	0.42	4¼ max 5 min 3	5 max 6 min 4
	8	271	2:6:1	2	5-6	3-4	0.58	2 max 3 min 1½	4 max 5 min 3½
	10	275	2:2:1	2	5-6	2-3	0.68	1½ max 2 min 1	2½ max 3 min 2
3 months	3	279	2:1	2	5-6	3-4	0.29	3½ max 5 min 2	½ max 1 min 0
	5	283	2:2:1	2	5-6	3-4	0.39	6 max 8 min 5	5 max 6½ min 3½
	8	287	2:1	2	5-6	3-4	0.82	2 max 2½ min 1	4 max 5 min 3
	10	291	2:2:1	2	6-7	4	0.72	3½ max 4 min 3	2½ max 3 min 2*
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)

\* Papilloma noted after five weeks of painting

followed for ten days were the two tongues advancing from both margins of the wound just about ready to join The thickness of the tongues increased during the first week from two rows of cells to four or five, after which it remained stationary or showed a decline to two to four rows after ten days (table 1, column 7)

Three days after excision there was scarcely any mitotic activity in the tongue, the mitotic count being about one fourth of that in normal resting epithelium The number of mitoses, however, rose during the next two days and reached a peak of five times the normal (mean) five days after operation Subsequently the mitotic count dropped gradually, but even ten days after excision the mean number of mitoses was still two and nine-tenths times higher than usual, the maximum being four and a half times and the minimum twice the normal (table 1, column 10)

five days after operation (table 2, column 6) The basal cells of this epithelium were 10 to 20 per cent larger than the hypertrophic basal cells at the margin of the wound in untreated animals Their mean mitotic count reached a peak of eight times the normal five days after excision (table 2, column 9) This compares with the maximum of six times (mean) the normal found in untreated controls Subsequently the number of mitoses dropped sharply After eight days the mean was only twice the normal, whereas in the control mice the number of mitoses ranged from twice to three and a half times the normal Thus in the benzene-treated mice as compared with the controls there was an early intensification of mitotic proliferation, but the return to the resting state was somewhat more rapid than in the latter

(b) *New Epithelium* In the benzene-treated mice the epithelial tongues advanced more rapidly toward the center of the wound than those in the untreated mice (table 2, column 8). Three days after operation the mean length of the tongues was 0.39 mm in the 2 benzene-treated mice, whereas in the 4 untreated mice it was only 0.29 mm. Five days after excision the corresponding mean figures were 0.57 mm in the benzene-treated mice and 0.46 mm in the 4 untreated animals. After eight days the tongue was 0.68 mm long in one of the benzene-treated mice, while in the other both tongues had already met in the center, indicating complete epithelization of the defect. In none of the untreated animals had the tongues met at this stage, the mean length of the tongues being 0.67 mm. Ten days

normal in the benzene-treated animals, compared with two and a half to four and a half times the normal in the untreated mice (table 2, column 10). Thus, also in the regenerating epithelium the return to the resting state was accelerated in the benzene-treated mice.

*Second Series Group 2* (benzene applied for two and for three months previous to the making of the wound)

—(a) *Old Epithelium* The structure of the epidermis at some distance from the wound as well as near the line of excision did not differ significantly from that seen after application of benzene for shorter periods. The number of mitoses rose and fell about as steeply as after two weeks or one month of treatment, or perhaps even somewhat more steeply inasmuch as the peaks were already present after three days and the drop was

TABLE 2—Benzene-Treated Animals

Duration of Treatment	Days After Excision	Animals	Ratio of Basal to Spinous Cells	Rows of Cells in			Length of Tongue, Mm	Mitosis in Multiples of the Normal Mean, Maximum and Minimum Values	
				Distant Epithelium	Insertion of Tongue	Tongue		Original Epithelium	New Epithelium
2 weeks	3	232	1:1	3	4:5	2:3	0.39	5 max 6 min 4	5 max 6 min 4
	5	236	1:1	3	8:9	4:5	0.56	8 max 10 min 6	5 max 6 min 5
	8	240	1:0.75	3	7	3:4	0.68	2 max 2 min 2	2 max 2 min 2
	10	244	1:1	3	7:8	3:4	Closing	1½ max 3 min 1	2 max 2½ min 1½
1 month	3	248	1:1	3	6:7	3:4	0.39	5 max 6 min 4	3½ max 4 min 2½
	5	325	1:1.5	2:3	5:6	2:3	0.58	6 max 7 min 4½	7 max 7½ min 6
	8	326	1:5:1	3	6:7	3:4	Closing	1½ max 2 min 1	3½ max 4 min 3
	10	327	1:2:1	2:3	5:6		Closed	1 max 1½ min 1	2 max 2½ min 1½
2 months	3	264	1:1:1	3	6:7	4:5	0.37	7 max 10 min 6	5½ max 6½ min 4
	5	268	1:2:1	3:4	6:7	3:4	0.72	4 max 5 min 3	4½ max 5½ min 4
	8	272	1:1:2	3	6:7	3:4	0.63	3 max 4 min 2½	2 max 2½ min 1½
	10	276	1:5:1	2:3	6:7		Closed	2 max 3 min 1½	1 max 1½ min 1
3 months	3	280	1:1	3	6:7	4	0.37	5 max 6 min 3	5 max 6 min 3½
	5	284	1:1	3:4	7:8	4:5	0.67	3 max 4 min 2	4½ max 5 min 4
	8	288	1:1:2	3	6:7		Closing	1½ max 2 min 1	2 max 3 min 1
	10	292	1:1	3	4:5		Closed	1½ max 3 min 1	2 max 3 min 1
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)

after operation the wounds were closing or closed in the benzene-treated mice, whereas in only 1 of the 4 untreated animals had the tongues approached each other closely. The thickness of the epithelial tongues resembled that found in the untreated controls. As in the latter, the tongues were composed of from two to five rows of cells (table 2, column 7).

The number of mitoses in the outgrowing epithelium was markedly increased over that found in the untreated controls. Three days after excision the mean number of mitoses was three and a half to five times the normal, whereas it was below normal in the untreated mice. After five days the mean mitotic count was five to seven times the normal, compared with four to six times in the untreated mice. After eight and ten days the mean of the number of mitoses had dropped to twice the

noticeable as early as five days after operation (table 2, column 9).

(b) *New Epithelium* The growth processes were similar to those seen after two weeks or one month of painting with benzene, no further intensification had taken place after two or three months' application of the substance. The lengths of the tongues as well as the numbers of mitoses were similar to those found after treatment with benzene for shorter periods (table 2, columns 8 and 10).

*Third Series Group 1* (methylcholanthrene-treated mice, methylcholanthrene applied for two weeks and for one month previous to the making of the wound)

(a) *Old Epithelium* The epidermis at a distance from the defect was markedly thickened and keratinized. Pearls of keratin filled the hair follicles, and frequently



the hair had fallen out. Here and there the nuclei of the epithelial cells had undergone pyknosis, karyolysis and karyorrhexis. Foci of polymorphonuclear and mononuclear leukocytes were found in the epidermis and in the subcutaneous tissue. In the latter, there was edema, and the collagenous fibers were fragmented. The epidermis was composed of three to five rows of cells, compared with two to three in mice painted with benzene alone (tables 2 and 3, column 5). The ratio of basal to spinous cells had shifted markedly in favor of the latter (table 3, column 4).

At the margin of the wound the thickness of the epidermis varied from four to ten rows during the period of observation. It was thus somewhat thicker than in the mice painted with benzene, which showed during the corresponding period variations of from four to nine rows of cells (tables 2 and 3, column 6). The basal cells were as hypertrophic as after treatment with benzene alone. Likewise, after two weeks of treatment with methylcholanthrene, the rise of the mitotic count was of the same order as in the benzene-painted animals (tables 2 and 3, column 9). However, after one month of application of methylcholanthrene the return to the resting state during the later stages of wound healing was delayed as compared with the course of events in both the normal and the benzene-painted mice. Ten days after excision, the mean of the mitotic counts still remained four to six times higher than normal, whereas in the benzene-treated and the untreated control mice the number of mitoses had dropped to almost normal (tables 1 to 3, column 9).

(b) *New Epithelium* Three days after operation the epithelial tongues advancing into the defect (table 3, column 8) were 0.40 mm (mean) long in the mice painted for two weeks, they were only 0.345 mm (mean) long in the mice painted for one month. Five days after excision the values were 0.52 mm (mean) in the animals painted for two weeks and 0.345 mm (mean) in those painted for one month. Eight and ten days after the making of the wound the defects were epithelized to a similar extent in both the benzene-painted and the methylcholanthrene-painted group.

The tongues consisted of two to six rows of cells as compared with two to five rows in the benzene-treated mice. The regenerating epithelial cells were almost twice as large as resting epidermal cells. After application of the carcinogen for two weeks the mean values for the mitotic counts (table 3, column 10) were slightly higher in the methylcholanthrene series. This difference became more apparent after application of the carcinogen for one month. Moreover, whereas after painting with benzene the mean mitotic count had dropped to twice the normal ten days after excision, the mitotic count was four or five and a half times the normal in the mice that had been painted with methylcholanthrene.

*Third Series Group 2* (methylcholanthrene applied for two and for three months previous to the making of the wound)—(a) *Old Epithelium* The epidermis at a distance from the wound had undergone further thickening, the average thickness being five to six rows three and five days, and four to five rows eight and ten days, after excision. Large amounts of keratin had been laid down. The spinous cells outnumbered the basal cells now as much as 25:1 in 2 animals (table 3, columns 4 and 5). Regression of the epithelial cells was more marked, and infiltration by polymorphonuclear and mononuclear leukocytes was more widespread than previously. However, the hypertrophy of the epidermal cells was the same as that found after two weeks or one month of painting.

The thickness of the marginal epithelium varied during the period of observation from five to ten cell rows, averaging seven to eight rows throughout the ten days of observation. The number of mitoses was greatly increased, particularly after three months of painting, when the mean values reached a maximum of from nine and a half to eleven and a half times the normal (table 3, column 9). The mitotic count was independent of the stage of repair, remaining high throughout the period of observation.

(b) *New Epithelium* The regenerating epithelial tongues were definitely shorter in the mice painted with methylcholanthrene than in the benzene-painted and the untreated animals (tables 1 to 3, column 8). After two months of application of methylcholanthrene there was little outgrowth during the first three days following operation (mean, 0.075 mm). But this inhibition was only temporary, and at later stages the advancement of the tongues was faster. However, after three months of painting the tongues did not increase much in length during the time of observation, being 0.295 (mean) mm long three days after excision and 0.35 mm (mean) after ten days.

The thickness of these short tongues showed marked variations of from two to seven rows of cells (table 3, column 7). The degree of hypertrophy of the advancing epithelial cells was similar to that found after shorter periods of painting. The mitotic proliferation had increased to a mean maximum of eight and three-fourth times the normal (table 3, column 10). After two months of treatment the wounds closed ten days after excision. There was a maximum of mitotic activity after five days, followed by a slow drop during the next three days and a somewhat steeper decline during the last days of observation, at the time when the wounds closed. However, there was no return to the normal resting state, and in accordance with the increased number of mitoses in the original epithelium, the mitotic count remained three and a fourth times the normal (table 3, column 10). After three months of painting the mean mitotic count was seven times the normal three days subsequent to excision, and it remained high (six and a fourth to eight and a half times the normal) throughout the period of observation.

#### COMMENT

Repair in the skin of unpainted mice followed the course observed in previous investigations.<sup>1</sup> Wounds 3 mm in diameter at the time of excision were not completely closed after ten days. There was a distinct mitotic cycle typical of regenerating epithelium as shown by Loeb and co-workers.<sup>3</sup> In the original epithelium the mitoses increased at first more rapidly than those in the new regenerating epithelium. In both maximum was reached at five days, and then the mitotic count declined, but even after ten days the number of mitoses in the old epithelium was twice as high and the number in the regenerating epithelium three times as high as in the resting skin.

<sup>3</sup> Loeb, L. *Arch f Entwicklungsmech n d Organ* **24** 638, 1907. Loeb, L., and Addison, W. H. F. *ibid* **32** 44, 1911. Loeb, L., and Spain, K. C. *J Exper Med* **21** 193, 1915.

TABLE 3—Methylcholanthrene-Treated Animals

Duration of Treatment	Days After Excision	Animals	Ratio of Basal to Spinous Cells	Rows of Cells in			Length of Tongue, Mm	Mitosis in Multiples of the Normal Mean, Maximum and Minimum Values	
				Distant Epithelium	Insertion of Tongue	Tongue		Original Epithelium	New Epithelium
2 weeks	3	233	1 4 1	4 5	7	4	0 37	5 max 6 min 4	5 max 5½ min 4
	3	234	1 1 1	5	7 8	4 5	0 43	5½ max 6 min 4½	5 max 5½ min 4½
							Mean 0 40	Mean 5¼	Mean 5
	5	237	1 1	4	7 8	4	0 51	7 max 9 min 5½	6½ max 8 min 5
	5	238	1 1 2	4 5	8 9	4 5	0 53	6 max 7 min 5½	7 max 8 min 6½
							Mean 0 52	Mean 6½	Mean 6¾
	8	241	1 1 2	3 4	6 7	4 5	0 83	2½ max 3 min 2	3½ max 4 min 2½
	8	242	1 1 3	5	8 9	5 6	1 05	4 max 5½ min 3½	5 max 5½ min 4½
							Mean 0 94	Mean 3¼	Mean 4¼
	10	245	2 1	3	7 8	3 4	Closing	1½ max 2 min 1	1 max 1½ min 1
1 month	10	246	1 5 1	3	6	4	Closed	1½ max 1½ min 1½	1½ max 2 min 1
								Mean 1½	Mean 1½
	3	231	1 1	5	5-6	2 3	0 39	5 max 6 min 4½	2½ max 3 min 2
	3	249	1 1 2 5	3 4	6 7	3 4	0 30	4 max 5½ min 2½	2 max 3 min 1
							Mean 0 345	Mean 4½	Mean 2¾
	5	253	1 1 5	4 5	9 10	4 5	0 38	5 max 6½ min 3½	7 max 9 min 5
	5	254	1 1 3	4 5	8 9	3 4	0 31	6 max 6½ min 4½	6½ max 9 min 5
							Mean 0 345	Mean 5½	Mean 6¾
	8	257	1 1 1	3	4 5		Closing	7 max 9 min 5½	4½ max 5 min 3
	8	258	1 1 2	3 4	6-7		Closing	5½ max 6 min 3½	2½ max 3 min 2
2 months								Mean 6¼	Mean 3½
	10	261	1 1 2 3	4 5	5 6	3	0 69	6 max 6 min 6	5½ max 6 min 5
	10	262	1 1 1	3 4	4 5		Closed	4 max 5½ min 3½	4 max 5 min 3½
								Mean 5	Mean 4¾
	3	265 †	1 1 2	4 5	5-6	2 3	0 08	6½ max 8½ min 5½	2 max 2½ min 1½
	3	266 †	1 1	4 5	6	1 2	0 07	8 max 10 min 6	3½ max 4 min 3
							Mean 0 075	Mean 7¼	Mean 2¾
	5	269 †	1 1 5	5 6	7 8	2 3	0 57	6 max 7 min 5	7½ max 9 min 5
	5	270	1 2	6 7	7 8	1 2	0 41	10 max 12 min 8	10 max 12 min 8
							Mean 0 49	Mean 8	Mean 8¾
3 months	8	273	1 2	3	5 6	4 5	0 69	5½ max 6½ min 4	5 max 6 min 4½
	8	274	1 1 2 3	3 4	8 9	4 5	0 87	10 max 12 min 9½	8½ max 9 min 7
							Mean 0 78	Mean 7¾	Mean 6¾
	10	277	1 1 2	4 5	7 8		Closing	8 max 10 min 7	3½ max 4 min 3
	10	278	1 1 5	4 5	8		Closed	10 max 11 min 7	3 max 4 min 2½
								Mean 9	Mean 3¼
	3	281 †#	1 1 5	5 6	8 9	3 4	0 28	12 max 15 min 10	6 max 8 min 5½
	3	282 †	1 1 8	5-6	8 9	2 3	0 31	11 max 13 min 10	8 max 9 min 7
							Mean 0 295	Mean 11½	Mean 7
	5	250 †	1 1 5	5 6	6-7	4 5	0 42	7 max 9 min 6	5½ max 6½ min 4½
3 months	5	286 *	1 2 5	5 6	7 8	3 4	0 32	12 max 14 min 10	7 max 8 min 6
							Mean 0 37	Mean 9½	Mean 6¼
	8	289 §	1 2 2	5	7 8	3 4	0 29	12 max 14 min 11	7½ max 8 min 6½
	8	290 †	1 2 5	5 6	6 7	4	0 39	10 max 12 min 9	6½ max 7½ min 5
							Mean 0 34	Mean 11	Mean 7
	10	293 †	1 2 2	7 8	9 10	6 7	0 29	8 max 10 min 7	8 max 10 min 7½
	10	294 †	1 2	5 6	7 8	5 6	0 41	14 max 15 min 12	9 max 10 min 8
							Mean 0 35	Mean 11	Mean 8½
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)

\* Papilloma noted after five weeks of painting  
† Papilloma noted after six weeks of painting  
‡ Papilloma noted after eight to nine weeks of painting  
§ Papilloma noted after twelve weeks of painting  
# Papilloma developed at the line of excision

Our previous observations concerning the effect on the repair of wounds of benzene applied to the skin before operation could be confirmed. Skin thus treated was thicker and reacted to the stimulation exerted by the wound more rapidly and more intensely than did normal skin. The mitotic count rose more rapidly and to a higher level than that in unpainted animals, and the migrating of the epithelial cells into the defect

affected epithelization of the wounds to a varying degree depending on the duration of treatment previous to the making of the wound. After two weeks of painting, epithelization was accelerated over the normal, and the defects were closed after ten days of observation. This effect on epithelization was comparable to that exerted by benzene. The mitotic activity in the original epithelium of the methylcholanthrene-treated mice was increased in comparison with that observed in unpainted animals. In the new epithelium mitotic activity was likewise intensified as compared with that in either untreated or benzene-painted mice.

Chart 1 demonstrates the mitotic activity in the old and the new epithelium in normal mice and in animals painted with benzene and methylcholanthrene for two weeks. In all three groups the typical mitotic cycle existed in the old as well as in the new epithelium. There was an early rise of the mitotic activity, then a drop to normal or nearly normal.

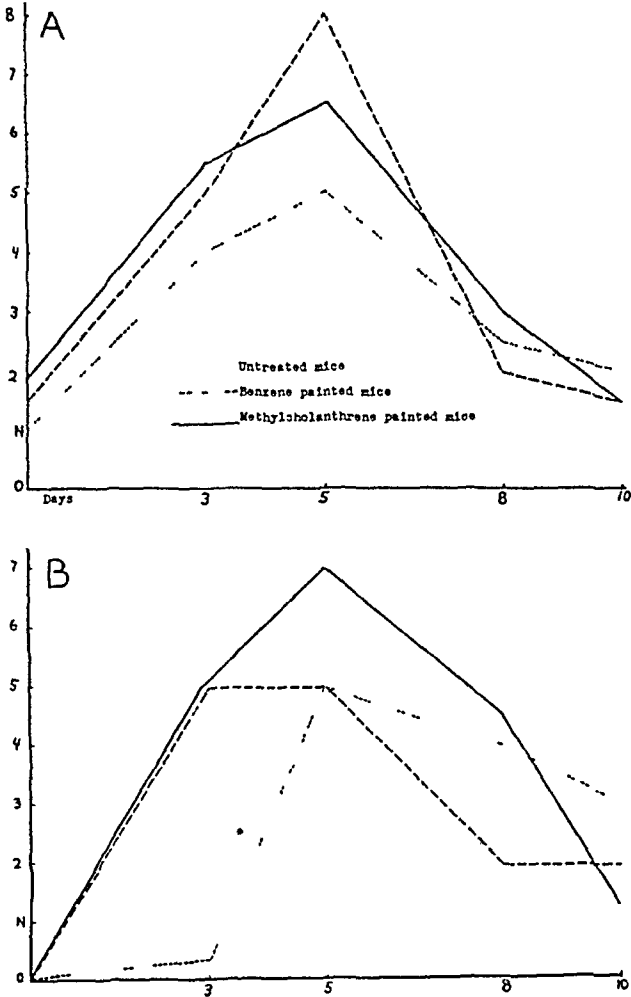


Chart 1—A, mitotic cycle in the original epithelium during ten days of wound repair in control mice and in animals treated for two weeks. B, mitotic cycle in the new regenerating epithelium. N stands for the normal value and the numbers above N for multiples of the normal value.

was accelerated over the normal. Consequently, epithelization of the wounds was hastened, and the drop of the mitotic activity, approximately coinciding with the closing of the wound (Loeb<sup>4a</sup>), was more rapid and reached a lower level in treated than in untreated mice. This stimulating effect of benzene was limited, however, and could not be intensified by prolonged application.

Methylcholanthrene dissolved in benzene and applied to the epidermis previous to excision

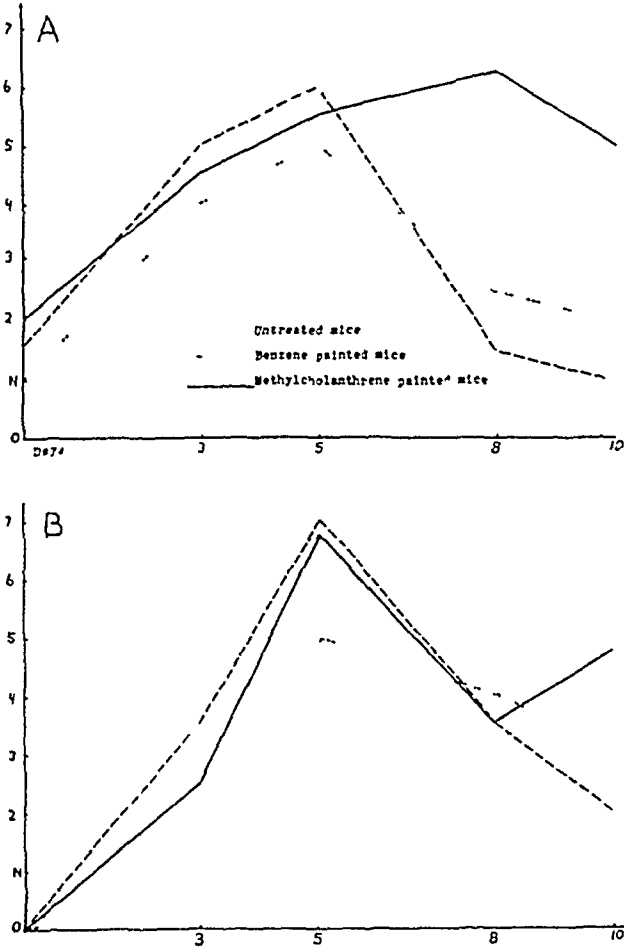


Chart 2—A, mitotic cycle in the original epithelium during ten days of wound repair in control mice and in animals treated for one month. B, mitotic cycle in the new regenerating epithelium. N stands for the normal value and the numbers above N for multiples of the normal value.

After one month of treatment with methylcholanthrene (chart 2) a change in the mitotic

4 (a) Loeb, L. J. M. Research 41 247, 1919. (b) Akaiwa, H. ibid 40 311 and 371, 1919.

cycle was noted in the old epithelium. While the absolute number of mitoses did not exceed that seen after two weeks of painting, there was no marked decline in the proliferative activity ten days after excision. This deviation from the ordinary cycle was especially striking in 3 of 4 mice in which the epithelial tongues had met and closed the defect, an occurrence which under normal conditions coincides with a steep drop of the number of mitoses. In the new epithelium, on the other hand, the mitotic cycle was still present, the sharp drop between the fifth and the eighth day being due possibly to some unexplained accidental factors.

In the mice painted for two months with methylcholanthrene (chart 3), the disturbance of the cycle of mitotic proliferation as shown in the lack of decline in the mitotic activity in the old epithelium during the second part of the cycle became more conspicuous. However, the migration of the epidermal cells into the wound was extremely slow during the first three days. This lag of cell movement was even more striking since the number of mitoses was higher than after two weeks or one month of painting. Closure of the wound occurred after ten days of observation at the same time as after two weeks of treatment. In view of the intense mitotic activity present after two months of painting, the epithelization of the defect was comparatively delayed, indicating beginning dissociation of cell proliferation and cell migration. The mitotic proliferation in the new epithelium continued to show the typical cycle: an early rise which was greater than that observed in controls or in benzene-treated mice, followed by a decline during the later stages of wound healing.

After three months of treatment with methylcholanthrene previous to excision (chart 4) the mitotic activity no longer revealed a cyclic character. The mitotic count in the old epithelium was high at the beginning and remained high throughout the period of observation, migration of the epithelium, on the other hand, made scarcely any progress after the first three days. The dissociation of cell movement and cell proliferation was now pronounced. Besides, there was a marked change in the mode of reaction of the new epithelium. Whereas, after shorter periods of painting the regenerating epithelium exhibited a rhythmic cycle during the course of repair, it failed to do so after three months of treatment. This seemed even more remarkable since the newly formed epithelium was at no time under the direct effect of the carcinogenic substance. This lack of a cyclic decline may perhaps be explained partly by the failure of the wounds to close, but probably it is largely due to the effect of methylcholanthrene.

A comparison of the course of healing of wounds subsequent to painting with benzpyrene and with methylcholanthrene revealed the following facts. At any given stage the keratinization of the epithelium was more marked under the influence of benzpyrene than under that of methylcholanthrene, regressive changes and inflammatory reaction in the epidermis and in the connective tissue were likewise more pronounced in the benzpyrene series. After two weeks of painting with benzpyrene, the stimulation of proliferation

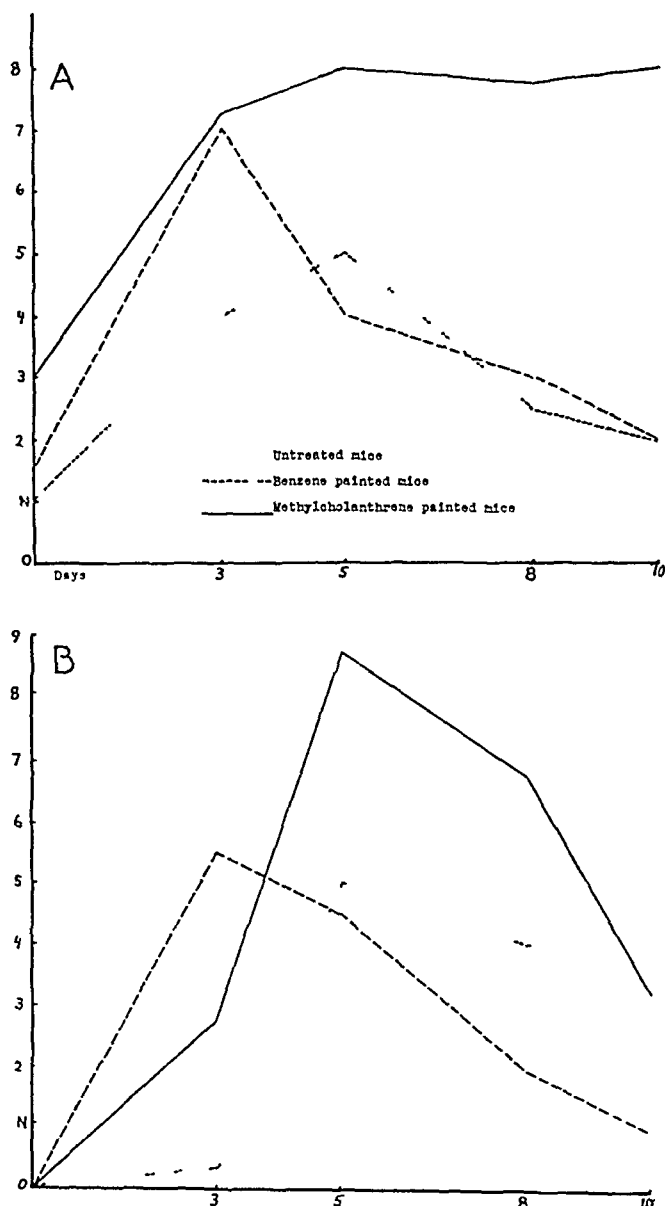


Chart 3—A, mitotic cycle in the original epithelium during ten days of wound repair in control mice and in animals treated for two months. B, mitotic cycle in the new regenerating epithelium. N stands for the normal value and the numbers above N for multiples of the normal value.

was somewhat more intense, and the mitotic counts rose slightly more rapidly than after the application of methylcholanthrene. On the other hand, the rhythmic cycle of epithelial proliferation in the mice painted with methylcholanthrene was more similar to that seen in the un-

treated series than to that in the benzpyrene group. After one month of treatment the intensification of proliferation and the cycle of mitotic activity took a similar course in both experimental groups. After two months of painting there was marked inhibition of the migration of epithelial cells into the wounds in the benzpyrene group, whereas in the methylcholanthrene series the inhibition of cell move-

however, the inhibition of cell migration was marked in both the benzpyrene-treated and the methylcholanthrene-treated animals.

The effect of methylcholanthrene on regenerating epithelium may thus be considered as more graded than that exerted by benzpyrene. This effect may be correlated with the observations of Nicod and Regamey,<sup>5</sup> who found that methylcholanthrene produced fewer carcinomas than benzpyrene in equally strong solution. In our animals, we noted the appearance of carcinoma after ninety days of painting with benzpyrene but we did not observe at that time a single carcinoma in mice treated with methylcholanthrene. However, papilloma seemed to develop more readily under the influence of methylcholanthrene than under that of benzpyrene.

The dissociation of cell migration from proliferation in the course of regeneration of the epidermis is not an effect of benzpyrene alone, it is also an effect of methylcholanthrene and perhaps of other carcinogenic agents. This does not imply, however, that it is necessarily correlated to the ability of these substances to produce cancer. As discussed previously the increased keratinization occurring as a consequence of the intensified proliferation of the epithelium or of changes in the base of the wound may play a role in the disturbance of the motility of the cells. Experiments are in progress to analyze this phenomenon further.

#### SUMMARY

Methylcholanthrene applied to the epidermis of mice before the making of a wound influences the course of regeneration in a way similar to benzpyrene. The application of methylcholanthrene for two weeks or for one month intensifies proliferation and migration of the epithelial cells and thus hastens the healing of the wound. But after three months of treatment with methylcholanthrene, cell migration is markedly inhibited and wound healing delayed in spite of the greatly increased proliferation of the epidermal cells. Between these two phases there is an intermediate stage in which the effect of methylcholanthrene differs from that of benzpyrene so far as the inhibition of cell migration is only transitory in the case of methylcholanthrene and is subsequently compensated for by rapid epithelization of the wound. The effect of methylcholanthrene on healing of wounds is thus a more quantitatively graded one than that of benzpyrene.

<sup>5</sup> Nicod, J. L., and Regamey, J. *Bull. Assoc. franç. p. l'étude du cancer* 27: 706, 1938.

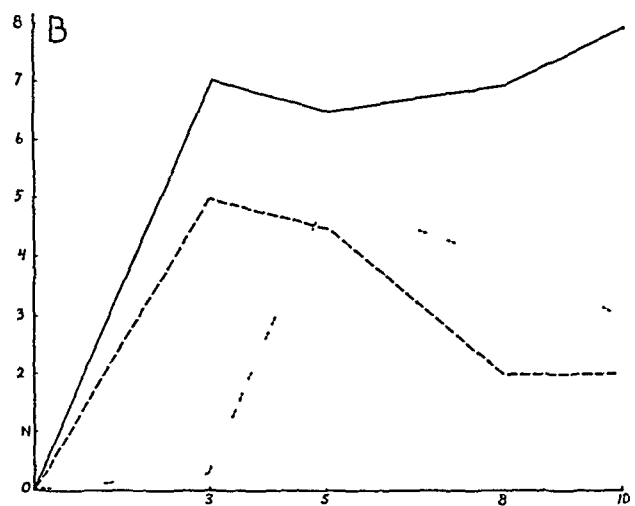
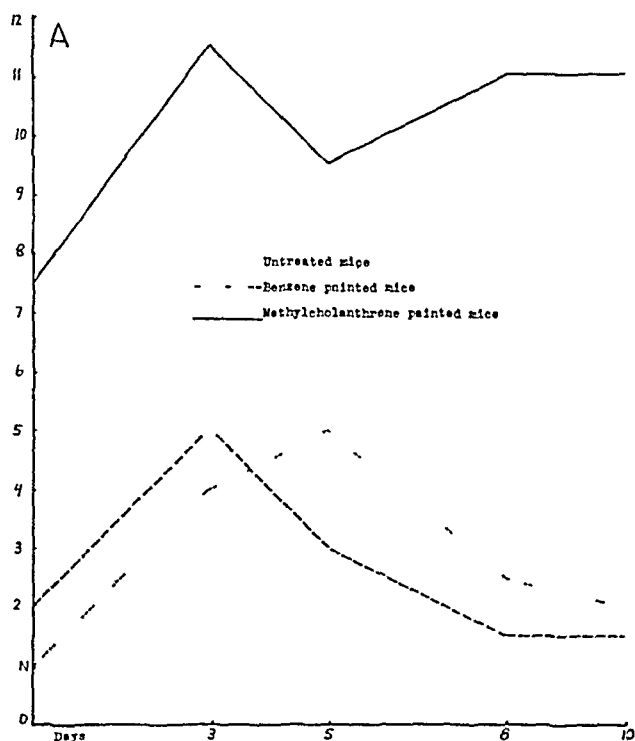


Chart 4—A, mitotic cycle in the original epithelium during ten days of wound repair in control mice and in animals treated for three months. B, mitotic cycle in the new regenerating epithelium. N stands for the normal value and the numbers above N for multiples of the normal value.

ment was relative rather than absolute. In the latter group the progress of epithelization was not commensurate with the increased rate of cell proliferation. After three months of treatment,

# A PRESACRAL CYST APPARENTLY ARISING FROM THE NEURENTERIC CANAL IN A NEWBORN INFANT

W W BRANDES, M.D., AND JOHN B SUTTON, M.D

NEW YORK

Anomalous developments and tumors not infrequently occur in the sacrococcygeal region. Tumors are relatively rare in the newborn. Tumor ventral to the sacrum was found by Calbet,<sup>1</sup> who collected 203 cases, to occur once in 34,582 births. Whittaker and Pemberton<sup>2</sup> stated that the incidence is 1 in every 40,000 registrations at the Mayo Clinic, reporting 22 cases.

This region passes through complicated phases of embryologic development. Hundling<sup>3</sup> stated that while the entoderm is forming the caudal intestine, the dorsal cord and the dorsal canal, the mesoderm the connective tissue, the blood vessels, the vertebrae and the muscles, and the ectoderm the primitive streak, the medullary tube and its vestiges, there is a continuation between the central canal of the spinal cord and the primitive alimentary canal around the caudal extremity of the notochord. This communication between the cord and the gut is known as the neurenteric canal. When the proctodeum, or primitive anus, invaginates to form part of the cloacal chamber, it meets the gut some distance anterior to and above the point where the neurenteric canal opens into it. Hence there is for a time a segment of intestine behind the anus termed the postanal gut. This, as well as the neurenteric canal, later becomes obliterated. The development of the urogenital tract is closely allied with these changes.

Guis and Stout<sup>4</sup> stated that the neurenteric canal is highly transitory in man and normally disappears before the neural groove is converted into a closed tube. Eternod<sup>5</sup> described a 21 mm embryo in which the neurenteric canal was observed.

Middeldorpf<sup>6</sup> in 1885, described tumors occurring in this region, and the term Middeldorpf tumors has commonly been applied to them since then. Whittaker and Pemberton<sup>2</sup> expressed the opinion, however, that this term should more correctly be limited to the teratoid lesions which arise from the postanal gut.

According to Ewing,<sup>7</sup> the embryologic structures which give rise to these growths are chiefly the fovea coccygea and coccygeal vestiges of the neurenteric canal, the postanal gut, the neurenteric canal and the proctodeal membrane. The structure of many of these tumors suggests origin from various sources such as the coccygeal body, bone, cartilage and the neurenteric canal rather than from the postanal gut. Various descriptive terms have been applied to tumors occurring in this region both in children and in adults: "teratoma," "dermoid cyst," "Middeldorpf tumor," "meningeal cyst," "ependymal glioma," "neuroblastoma," "chondroma," "giant cell tumor," "myoma," "fibroma," "lipoma," "cloacal vestiges," "parasitic fetus," "sarcoma," "carcinoma," and "endothelioma." A large proportion are mainly cystic in character, while others are solid, with small cysts scattered throughout.

Teratoma arising in the coccygeal region and having cysts lined with neural elements has been described by Raven<sup>8</sup>, Engelmann,<sup>9</sup> Scheuermann,<sup>10</sup> Pearse<sup>11</sup> and Law<sup>12</sup>. Hundling<sup>3</sup> reported 5 cases of presacral tumor of the adult, in these cases the tumor was composed of nerve tissue and was diagnosed as ependymal glioma. Guess<sup>13</sup> described a tumor that involved presacral and postsacral regions in an infant and diagnosed it as neurocarcinoma. Metastases were present in the inguinal nodes. He assumed that

From the Department of Pathology, Roosevelt Hospital.

1 Calbet, J. Contribution a l'etude des tumeurs congenitales d'origine parasitaire de la region sacrococcygienne, Paris, G. Steinheil, 1893.

2 Whittaker, L. D., and Pemberton, J. de J. Ann Surg **107** 96, 1938.

3 Hundling, H. W. Surg, Gynec & Obst **38** 518, 1924.

4 Guis, J. A., and Stout, A. P. Arch Surg **37** 268, 1938.

5 Eternod, A. C. F. Anat Anz **15** 181, 1898.

6 Middeldorpf, K. Virchows Arch f path Anat **101** 37, 1885.

7 Ewing, J. Neoplastic Diseases, ed 4, Philadelphia, W. B. Saunders Company, 1940, p 1065.

8 Raven, R. W. Brit J Surg **23** 337, 1935.

9 Engelmann, Arch f klin Chir **72** 942, 1903.

10 Scheuermann, H. Arch f klin Chir **88** 310, 1908-1909.

11 Pearse, H. E. Surg, Gynec & Obst **33** 164, 1921.

12 Law, A. A. Surg, Gynec & Obst **17** 340, 1913.

13 Guess, H. C. Tr Am Proct Soc **37** 113, 1936.

it arose from the neurenteric canal. Mallory<sup>14</sup> reported a glioma situated in the postsacral region. Peyron<sup>15</sup> maintained that no genuine vestiges of cysts arising from the neurenteric canal had ever been described.

We wish to report a cyst lined with glia and ependymal cells which occurred in the caudal area of a newborn child.

A girl 3 days of age was admitted to the Roosevelt Hospital as a patient of Dr. James Thompson. A large



Fig. 1—Anterior view of a cyst situated posterior to the anus.

Fig. 2—Section from the wall of the cyst ( $\times 150$ ). Note the dark row of ependymal cells on the surface of the lining, resting on a narrow zone of glia. The glia extends for short distances into the stroma.

Fig. 3—Cajal's silver stain demonstrating well formed astrocytes ( $\times 600$ ).

<sup>14</sup> Mallory, F. B. *J. M. Research* **13** 113, 1904-1905.

<sup>15</sup> Peyron, A. *Bull. Assoc. franç. p. l'étude du cancer* **17** 613, 1928.

cystic mass was present in the sacrococcygeal region. Prenatally, because of the large size of the mother's abdomen, it had been suspected that twins were present, but roentgen examination had revealed only one fetus and had given no evidence of the cyst. The latter had



not interfered with labor, although at the time of birth it was the size of a small grapefruit. Within the three days following birth it had increased to the size of the child's head. Urination and defecation were not interfered with (fig 1).

The infant was well developed and normal except for the cyst. The skin was tightly stretched over the mass, which transilluminated well. It lay posterior to the rectum and extended into the pelvis, and the sacrum and the coccyx could be palpated on its posterior aspect. Compression of the cyst did not cause ballooning of the fontanels. Neurologic examination disclosed no abnormality.

Roentgen films showed a mass in the region of the perineum and the buttocks. The sacrum and the coccyx were underdeveloped, and the last two segments were displaced posteriorly. Along what appeared to be a septum were minute deposits of calcium.

Laboratory studies of the blood and the urine gave negative results.

At operation, the cyst was found to be rather firmly attached to the posterior aspect of the anus and distal part of the rectum and to the anterior aspect of the sacrum. It was removed intact, and the wound was closed primarily. Infection of the wound complicated the postoperative course, but the child was discharged on the forty-sixth day with complete healing.

Pathologic examination revealed a cystic mass measuring 10 by 12 cm and weighing 340 Gm. Approximately half of its surface was covered with smooth thin skin. It transilluminated throughout. It contained slightly turbid watery fluid with a faintly greenish tinge. The lining was everywhere smooth and gray.

The fluid contained 0.2 per cent protein, and its  $pH$  was 7.8, the amylase value was 51 units, the sugar content was 60 mg per hundred cubic centimeters.

Microscopic sections revealed a thin covering of skin with short rete pegs and slight keratinization of the surface layers of the epidermis. The structure of the corium was normal. Sections cut from multiple areas showed a thin lining layer of glial tissue (fig 2) with short extensions into the surrounding fibrous stroma. The glia was everywhere fully differentiated. Cajal's stain (fig 3) showed well developed astrocytes and fibrillary stroma. In many areas cells perpendicularly arranged rested on this underlying glial tissue.

#### COMMENT

The case is unique in that the cyst was lined throughout with well differentiated glial tissue, everywhere uniform in thickness. The fact that ependymal cells were present only in scattered areas can be explained on the basis of the rapid enlargement of the cyst following birth, which left only scattered remnants of the ependymal lining as the cyst wall was stretched. This rapid enlargement must have been due in part at least to the liberation of the infant with the cyst from the confined uterine space. Clinical examination produced no evidence of a connection with the central nervous system, and postoperatively no leakage of spinal fluid occurred.

The structure of the cyst wall and its position anterior to the sacrum indicate that it arose from the neurenteric canal. The main element of the wall was the neural tissue. The only mesodermal element present was the stroma supporting the blood vessels and the glial lining. In the areas examined, no muscle, bone, cartilage or epithelial elements were seen. It is therefore a simple cyst of neural origin and cannot be classified as a teratoma. Hansmann<sup>16</sup> expressed the opinion that frequently when a remnant of the neurenteric canal is the source of a tumor, there is an anterior sacral defect. In this case the sacrum was underdeveloped.

#### SUMMARY

A presacral cyst with a lining composed of fully differentiated glial and ependymal tissue was surgically removed from a newborn infant. Its structure and position indicated origin from the neurenteric canal.

<sup>16</sup> Hansmann, G. H. Surg., Gynec. & Obst. **42**: 124, 1926.

CORONARY ARTERIOSCLEROSIS AND MYOCARDIAL INFARCTION  
AS STUDIED BY AN INJECTION TECHNIC

J B HOLYOKE, M D  
ROCHESTER, MINN

A more accurate concept of the significance of sclerosis of the coronary arteries in relation to myocardial disease has been achieved in recent years. The development of such a concept has been aided by an injection and dissection technic devised by M J Schlesinger<sup>1</sup>

Between July 14, 1943 and May 18, 1944, adult hearts from the autopsy service of the Mary Hitchcock Memorial Hospital, Hanover, N H, were examined by this technic. After injection of the vessels and roentgen examination of the heart, the coronary arteries were dissected, and all occlusions and narrowings were recorded as to location and length of the portion of vessel involved. In narrowing without occlusion, the degree of constriction was estimated. Slight changes were designated as grade I and extreme changes as grade IV. Anastomoses were likewise recorded.

distribution of the occlusions in age and sex groups is indicated in table 1

TABLE 1—Distribution of Occlusions of the Coronary Arteries in Age and Sex Groups

Age Group	Males	No with Occlusions	Females	No with Occlusions	Total No of Persons	No with Occlusions
20-29	1	0	1	0	2	0
30-39	2	0	2	0	4	0
40-49	1	0	4	0	5	0
50-59	0	1	6	1	15	2
60-69	11	2	8	1	19	3
70-79	10	3	7	2	17	5
80-89	3	1	1	1	4	2
90-99	0	0	1	0	1	0
Total	40	7	30	5	70	12

In the 12 hearts with occlusions, thirty-one points of obstruction were found. Eight of these were recent, and twenty-three were old. The recent obstructions were all produced by throm-

TABLE 2—Distribution of Occlusions in the Three Systems of the Coronary Arteries

	Left Descending			Left Circumflex			Right			Total
	Stem	Branch	Total	Stem	Branch	Total	Stem	Branch	Total	
Recent	4	2	6	0	1	1	1	0	1	8
Old	3	8	11	1	4	5	4	3	7	23
Total	7	10	17	1	5	6	5	3	8	31

The myocardium was examined by splitting the left ventricle from apex to base parallel to the pericardium, thus exposing the largest possible surface of the myocardium in one plane. The septum was similarly split. Other incisions were made perpendicular to the first to reveal lesions lying in other planes. Recent infarcts and scars were described. From ten to twenty sections representing all regions of the heart were taken for microscopic study. The observations were correlated with the clinical findings.

Occlusions of the Coronary Arteries

Seventy hearts were examined. In 12 the coronary arteries were seen to be occluded. The

basis with or without intimal hemorrhages and were all estimated as less than two weeks old. All remaining occlusions were designated as "old occlusions." Horn and Finkelstein<sup>2</sup> pointed out the difficulty of separating the lesions into those due to old acute occlusions and those due to gradual atherosclerotic narrowing.

Multiple complete obstructions in single cases were seen. In 1 case there were one fresh and five old occlusions. In a second there were four recent and three old occlusions. The average length of the portion of vessel involved in old lesions was 0.3 cm. Seven involved portions measured 0.2 cm or less. Thirteen points of obstruction were in the three main stems and eighteen in their branches. The system of vessels most often involved was the left descending coronary artery and its branches (table 2).

From the Department of Pathology, Dartmouth Medical School, and the Laboratories of the Mary Hitchcock Memorial Hospital, Hanover, N H

1 Schlesinger, M J. Am Heart J 15:528, 1938

2 Horn, H, and Finkelstein, L E. Am Heart J 19:655, 1940

# INTERARTERIAL ANASTOMOSIS

Three of 11 hearts with old occlusions showed no gross scars (table 3). Two of 5 hearts with recent occlusions of main stems showed no recent macroscopic infarcts (table 7). Occlusion without infarction can be explained only in the presence of a rich collateral circulation. Anastomoses were demonstrated in all hearts with severe coronary arteriosclerosis. Among the remaining hearts, collateral circulation was indicated only in several instances of marked cardiac hypertrophy.

## OLD OCCLUSIONS WITHOUT INFARCTS OR COMPLAINTS REFERABLE TO THE HEART

Three patients in the group of 12 with occlusions gave no history of complaints referable to

sclerosis or in whom there was an increased demand for blood supply in the myocardium, the inadequacy of the coronary circulation was indicated clinically during increased activity by angina pectoris. This insufficiency of the coronary circulation was only relative and was relieved on resumption of rest. The data on 9 patients with angina pectoris are summarized in table 4. When sclerosis was slight, other factors decreasing coronary flow or calling for increased cardiac activity were found as abnormalities. In the smallest heart, two main trunks were completely occluded, and all three main stems showed extreme narrowing. In the eighth patient only one main stem was involved in marked narrowing, but long-standing extreme hypertension with prolonged cardiac decompensation was present. In the ninth patient there

TABLE 3—Data on Patients Who Had Old Occlusions Without Scars

Age of Patient	Sex	Heart Weight, Gm	Estimated Maximum Normal Heart Weight * Gm	Blood Pressure		Occlusions	Systems with Grade III or IV Narrowing
				Systolic	Diastolic		
74	M	390	373	115	75	2 stems	3
67	F	270	250	180	90	2 branches	3
83	F	386	268	170	82	2 branches	1

\* According to H. L. Smith (Am Heart J 4: 79, 1928).

TABLE 4—Data on Nine Patients with Angina Pectoris

Age of Patient	Sex	Heart Weight, Gm	Estimated Maximum Normal Heart Weight, Gm	Blood Pressure		Old Occlusions		Systems with Narrowing of Grade III or IV	Complicating Lesions	Macroscopic Scars
				Systolic	Diastolic	Stems	Branches			
56	F	340	366	170	90	2	1	3		+
52	M	400	356	150	90	1	1	3		+
63	M	470	322	170	90	1	0	3		+
69	M	470	372	160	90	1	0	3		+
71	M	400	368	180	110	1	0	2		+
78	M	500	310	190	100	0	2	3		+
73	M	540	370	130	80*	0	0	3		+
49	F	583	268	280	160	0	0	1		+
54	M	530	310	100	85	0	0	0	Aortic stenosis	?

\* Recorded in the presence of fibrillation and extreme cardiac decompensation.

the heart. In their myocardiums no areas of grossly visible scarring were seen (table 3). Coronary arteriosclerosis was severe, however, only 1 of the hearts was of markedly increased size, the heart with the least sclerosis of the coronary arteries. In only 1 case was a moderately high blood pressure recorded, the case in which the smallest heart was noted. Factors requiring an increased blood supply were generally slight, and there was no element apparent other than arteriosclerosis to limit the flow of blood through the coronary arteries.

## ANGINA PECTORIS

In the group of patients (table 4) in whom there was an extreme degree of coronary arterio-

was only slight sclerosis of the coronary vessels. A stenosed aortic valve, however, might have served to decrease the coronary blood flow and, at the same time, to increase the demand on the myocardium.

## MYOCARDIAL INFARCTION

Myocardial anoxia, if severe and prolonged, may lead to infarction with typical symptoms and typical physical and laboratory findings. In table 5 are summarized data on 3 patients with acute occlusion followed by infarction. The occluding lesions in every instance proved to be thromboses with or without intimal hemorrhages. In all cases typical symptoms and signs of infarc-

tion were present The infarcts were irregular in outline and poorly demarcated

A striking feature in each instance was the extreme degree of arterial obstruction withstood by the heart before its circulation became so limited as to result in changes incompatible with life Necrosis of a large area of heart muscle most commonly follows acute occlusion of a coronary artery, but similar changes are seen in the absence of recent total arterial obstruction Summarized in table 6 are the data on a patient who had infarction without occlusion Ten weeks before death he had severe substernal pain, the duration was approximately three and one-

preferred the diagnosis "acute coronary insufficiency"

This case supports the thesis that the diagnosis "acute coronary occlusion" can be made logically only when characteristic symptoms are initiated under conditions in which there has not been a call for increased supply of blood to the heart or in which other factors which might rapidly decrease the oxygen carried to the myocardium, such as shock and hemorrhage, are absent Furthermore, acute occlusion of the coronary arteries may occur without myocardial infarction Therefore, it is unwise to assume that infarction has occurred unless the telltale

TABLE 5—Data on Patients with Recent Occlusion Followed by Infarction

Age of Patient	Sex	Heart Weight, Gm	Estimated Maximum Normal Weight, Gm	Duration of Illness	Duration of Pain	Estimated Age of Infarct	Old Occlusions		Systems with Grades III or IV Sclerosis	Recent Occlusions	
							Stems	Branches		Stems	Branches
54	F	340	366	4 days	Prolonged	4 days	2	1	3	2	2
78	M	500	310	13 days	Prolonged	13 days±	0	2	3	1	0
74	F	400	226	10 hours	10 hours	Hours	0	5	3	1	0

half hours, the onset occurred during exertion No program of treatment was followed There were later episodes of "stinging chest pain" The patient died of general peritonitis A large

signs of infarcted heart muscle are encountered In table 7 are the data on 2 patients who had thrombosis of the coronary arteries without corresponding infarcts though pain, and in 1 patient shock strongly suggested myocardial infarction There was no friction rub, leukocytosis, fever or characteristic electrocardiographic picture of myocardial infarction Acute occlusions were present, but no grossly recognized infarcts were detected though the estimated age of these occlusions was such that necrosis of heart muscle could have occurred

TABLE 6—Data on a Patient Who Had Infarction Without Occlusion

Age of Patient	Sex	Heart Weight, Gm	Estimated Maximum Normal Weight, Gm	Blood Pressure		Systems with Grade III Sclerosis	Estimated Age of Infarct
				Systolic	Diastolic		
51	M	375	371	150	100	2	23 mo

TABLE 7—Data on Two Patients Who Had Recent Occlusion Without Recent Infarct

Age of Patient	Sex	Heart Weight, Gm	Estimated Maximum Normal Heart Weight, Gm	Blood Pressure		Duration of Thrombus	Recent Occlusion (Location)	Old Occlusion	Systems with Grade III or IV Narrowing
				Systolic	Diastolic				
63	M	470	322	170	90	Days	1 stem	0	3
53	M	400	356	150	90	Days	1 stem	2 stems	3

poorly demarcated myocardial infarct in the final stages of organization was observed This case cannot be labeled one of angina pectoris, as the pain was not relieved at rest but was prolonged It was not "coronary occlusion," as no occlusion existed Schlesinger and co-workers<sup>3</sup> have used the term "coronary failure" to describe such a condition, while Master and his colleagues<sup>4</sup> have

That large scars seen in the myocardium are almost universally the aftermath of infarction is generally accepted The cause of small scattered scars is less evident Brown<sup>5</sup> concluded that ischemia was the cause of most of the scarring in a group of 110 hearts with "myocardial fibrosis"

Grossly visible scarring was observed in 26 of the 70 hearts in the present series The scars ranged from those covering several square centimeters to those consisting of a few scattered areas

3 Blumgart, H L , Schlesinger, M J , and Zoll, P M J A M A 116 91, 1941  
4 Master, A M , Jaffe, H L , Dack, S , and Gershman, A (a) J Mt Sinai Hosp 8 820, 1942, (b) Am Heart J 27 803, 1944

5 Brown, M R Am J M Sc 184 707, 1932

of gray streaking Table 8 indicates the distribution of the lesions as to age and sex, and table 9 summarizes the anatomic or clinical abnormalities considered possibly responsible for the lesions

TABLE 8—*Distribution of Scars in Sex and Age Groups*

	20-39	40-49	50-59	60-69	70-79	80-89	90-99	Total
Males	0	0	5	7	6	1	0	19
Females	0	1	1	0	4	0	1	7

TABLE 9—*Possible Etiologic Factors in the Production of Myocardial Scars*

Etiologic Factors	Cases
Coronary arteriosclerosis with hypertension	12
Coronary arteriosclerosis without hypertension	4
Hypertension alone	4
Aortic stenosis	3
Episodes of severe hemorrhage from chronic duodenal ulcer	1
Hodgkin's disease	2

There were 16 hypertrophied hearts in this group, the enlargement of 13 was associated

focal myocardial scars seen in all but the sixth case In this group as in that in table 9 a variety of conditions were present to produce a discrepancy between the heart's need and the supply of oxygen .

A review of this study shows that among conditions accompanying myocardial scarring or infarction the most frequent is sclerosis of the coronary arteries However, often other abnormalities appeared simultaneously with or independently of arteriosclerosis It is thus indicated that coronary arteriosclerosis is only one of several factors which may act to produce myocardial anoxia

## COMMENT

Though many abnormalities frequently accompany scarring or infarction of heart muscle, clinicians and pathologists alike tend to consider only coronary arteriosclerosis Yet there is abundant evidence<sup>7</sup> that collateral circulation may develop rapidly and effectively to assume the function of the constricted vessels The

TABLE 10—*Data on Patients in Whom Focal Lesions of the Myocardium Had Occurred*

Age of Patient	Sex	Heart Weight, Gm	Estimated Maximum Normal Heart Weight, Gm	Old Occlusions	Recent Occlusions	Systems with Grade III or IV Narrowing	Healed Macroscopic Scars	Other Important Factors
79	M	555	333	0	0	0	+	Calcareous aortic valvular disease
50	M	1,040	412	0	0	0	+	Calcareous aortic valvular disease
74	F	400	226	Branches, 5	0	3	+	Hypertension
63	M	470	322	0	Stems, 1	3	+	Hypertension
52	M	400	356	Stems, 1, branches, 1	Stems, 1	3	+	Hypertension
71	F	296	193	0	0	0	0	Organizing pneumonia, pulmonary fibrosis

with hypertension and that of 3 with aortic stenosis All hearts listed as showing arteriosclerosis contained at least one main stem with sclerosis interpreted as grade III or IV Abnormalities were present in each heart of a type to either increase its need for blood or to decrease the oxygen carried to it by the blood Two cases of Hodgkin's disease were apparent exceptions Ten hearts of the group showed no significant arteriosclerosis

In table 10 are summarized the data on patients whose myocardium showed either microscopic zones of necrosis with acute inflammatory exudate or small areas of granulation tissue These lesions were histologically identical with larger obvious infarcts, differing from them only in size Such lesions in similar circumstances have been described by others<sup>6</sup> They were interpreted as minute infarcts, and it is believed that their end stage would appear similar to the

importance of this collateral flow is paramount in determining the effect of occlusive disease of the coronary arteries on the myocardium

As sclerosis of coronary arteries progresses and new anastomotic channels develop, the path of blood flow may become quite complex, so that areas once supplied by one artery may now be supplied in part or entirely by a different artery whose origin may be far removed from the first Further narrowing or sudden occlusion in this circumstance could be expected to render a different intensity and pattern of ischemia than

6 (a) Gross, H, and Spark, C Am Heart J 14 160, 1937 (b) Fahr, G J A M A 105 1396, 1935

7 (a) Lowe, T E Am Heart J 21 326, 1941 (b) Spalteholz, W Die Arterien der Herzwand, Leipzig, S Hirzel, 1924 (c) Gross, L The Blood Supply to the Heart, New York, Paul B Hoeber, 1921 (d) Blumgart, H L, Schlesinger, M J, and Davis, D Am Heart J 19 1, 1940 (e) Prinzmetal, M, Kayland, A, Margolis, C, and Tragerman, L J J Mt Sinai Hosp 8 933, 1942 (f) Wiggers, C J Am Heart J 11 641, 1936 (g) Blumgart, H L, Gilligan, D R, Zoll, P M, Freedberg, A S, and Schlesinger, M J Tr A Am Physicians 57.152, 1942 (h) Eckstein, R W, Gregg, D E, and Pritchard, W H Am J Physiol 132 351, 1941 (i) Schlesinger<sup>1</sup>

similar changes in a normal set of vessels. These differences are demonstrated in experimental and autopsy studies. Ligation of a coronary artery in a dog's heart produces a well circumscribed area of infarction.<sup>8</sup> Similarly it has been observed that recent large circumscribed infarcts in human hearts are usually the results of sudden occlusion in a set of relatively lightly involved vessels.<sup>9</sup> In contrast patchy necrosis or "infarction at a distance"<sup>10</sup> may occur in sudden arterial occlusion in hearts with severe coronary arteriosclerosis. At other times recent myocardial changes may be absent.<sup>11</sup> Lowe has shown by injecting dye into branches of sclerosed coronary vessels of human hearts that the distribution of stained myocardium is patchy. Many have noted patchy distribution of scarring in hearts with severe arteriosclerosis.<sup>12</sup>

Variations in the fundamental pattern of coronary arteries may further affect the result of occlusive disease. Several authors<sup>13</sup> have observed a reciprocal relationship in the distribution of the right and the left circumflex coronary arteries. Schlesinger<sup>13a</sup> divided hearts into three groups on this basis. He demonstrated that large anastomoses develop with varying facility according to the type of circulation present. Therefore, a given amount of sclerosis could be expected to produce varying amounts of damage according to the pattern of vessels present.

The ability of the coronary circulation to adapt itself readily to occlusive arterial disease accounts for the many observations that extreme arteriosclerosis to the point of occlusion may be found in hearts of persons who have never complained of angina pectoris or of symptoms of cardiac decompensation and in whose hearts no areas of scarring or evidences of failure are found. In such persons the coronary circulation supplies the heart muscle with sufficient oxygen to maintain it at rest and during the usual activity of the individual. This and other studies<sup>14</sup> indicate that this group of people

generally lack conditions other than coronary arteriosclerosis which might produce myocardial anoxia.

Such additional conditions indicated as important by clinical, pathologic and experimental studies can be divided into three groups: (1) increased oxygen requirement of the heart, (2) decreased oxygen-carrying function of the blood, (3) decreased volume flow of the coronary circulation.

The first condition may be produced by factors augmenting the heart's activity, such as hypertension,<sup>15</sup> thyrotoxicosis,<sup>16</sup> valvular heart disease<sup>17</sup> and physical exertion.<sup>18</sup> Hypertrophy of a heart requires an increased blood supply, and, according to some workers, is important in decreasing the vascular bed in relation to the muscle mass.<sup>19</sup> Also it may produce ischemia of the individual fiber by increasing the size of the fiber and thus the distance that oxygen must diffuse.<sup>20</sup> This thesis, however, is contested.<sup>21</sup>

A decrease in the oxygen-carrying function of the blood may occur in anemia,<sup>22</sup> carbon monoxide poisoning,<sup>23</sup> exposure to oxygen-poor atmospheres<sup>24</sup> and in certain pulmonary diseases.<sup>25</sup>

A decrease in volume of coronary circulation may occur in such conditions as occlusion of coronary ostia in syphilis,<sup>26</sup> rheumatic arteritis,<sup>27</sup> periarteritis nodosa,<sup>28</sup> myocardial arteriolar

8 Karsner, H. T., and Dwyer, J. E. *J. M. Research* **34** 21, 1916.

9 Lowe.<sup>7a</sup> Blumgart and others.<sup>7d</sup>

10 Bean, W. B. *Ann. Int. Med.* **12** 71, 1938. Blumgart and others.<sup>7d</sup>

11 Saphir, O., Priest, W. S., Hamburger, W. W., and Katz, L. N. *Am. Heart J.* **10** 567 and 762, 1935. Eckstein and others.<sup>7h</sup>

12 Friedberg, C. K., and Horn, H. *J. A. M. A.* **112** 1675, 1939. Master and others.<sup>4a</sup> Blumgart and others.<sup>7d</sup>

13 (a) Schlesinger, M. J. *Arch. Path.* **30** 403, 1940. (b) Whitten, M. B. *Arch. Int. Med.* **45** 381, 1930. (c) Gross.<sup>7c</sup>

14 Blumgart and others (footnotes 3 and 7 d).

15 Levine, V. *Arch. Path.* **18** 331, 1934. Fahr.<sup>6b</sup>

16 Menne, F. R., Jones, O. N., and Jones, N. W. *Arch. Path.* **17** 333, 1934.

17 Blumgart and others.<sup>3</sup> Friedberg and Horn.<sup>12</sup>

18 (a) Wood, F. C., and Wolfeith, C. C. *Arch. Int. Med.* **47** 339, 1931. (b) Riseman, J. E. F., and Brown, M. G. *Am. Heart J.* **18** 150, 1939.

19 Wearn, J. T. *Bull. New York Acad. Med.* **17** 754, 1941. Gross and Spark.<sup>6a</sup>

20 Harrison, T. R., Ashman, R., and Larson, R. M. *Arch. Int. Med.* **49** 151, 1932. Gross.<sup>6a</sup> Wearn.<sup>19</sup>

21 Dock, W. *J. Exper. Med.* **74** 177, 1941.

22 Buchner, F., and von Lucedow, W. *Beitr. z. path. Anat. u. z. allg. Path.* **93** 169, 1934. Master, A. M., and Jaffe, H. L. *J. Mt. Sinai Hosp.* **7** 26, 1940. Friedberg and Horn.<sup>12</sup>

23 Christ, C. *Beitr. z. path. Anat. u. z. allg. Path.* **94** 111, 1934.

24 Rothschild, M. A., and Kissen, M. *Am. Heart J.* **8** 729, 1933. *Proc. Soc. Exper. Biol. & Med.* **29** 577, 1932. Riseman and Brown.<sup>18b</sup>

25 Master, A. M., Gubner, R., Dack, S., and Jaffe, H. L. *Arch. Int. Med.* **67** 647, 1941. Blumgart and others.<sup>7d</sup> Friedberg and Horn.<sup>12</sup>

26 Clawson, B. J. *Am. Heart J.* **4** 1, 1928. Saphir, O., and Scott, R. W. *ibid.* **6** 56, 1930.

27 (a) Slater, S. *Am. J. M. Sc.* **179** 22, 1930. (b) Karsner, H. T., and Bayless, F. *Am. Heart J.* **9** 557, 1934. (c) Hall, E. M., and Ichiooka, T. *Am. J. Path.* **16** 761, 1940.

28 Ophuls, W. *Arch. Int. Med.* **32** 870, 1923. Arkin, A. *Am. J. Path.* **6** 401, 1930.

sclerosis,<sup>29</sup> aortic valvular disease,<sup>30</sup> cardiac decompensation<sup>31</sup> and certain arrhythmias<sup>32</sup>. Any condition provoking prolonged episodes of low blood pressure may be associated with infarction of heart muscle<sup>33</sup>. A derangement of reflex or hormonal control of coronary blood flow must be a significant element<sup>34</sup>. Experiments in animals<sup>35</sup> and studies of the human heart<sup>31</sup> lend abundant evidence to support this conclusion. For example, demonstrations of the effect of cold in angina pectoris are striking<sup>36</sup>. These factors may well account for discrepancies in symptoms or in myocardial changes in cases with similar anatomic pictures.

Observations from the study of this series and data collected from the literature indicate that coronary arteriosclerosis, though occurring most frequently, is only one of many possible abnormalities that may act to produce an insufficient supply of oxygen in the myocardium.

29 Lisa, J. R., and Brown, C. R. *Ann Int Med* **14** 2147, 1941. Plaut, A., and Kramer, M. L. *Arch Path* **22** 393, 1936.

30 Green, H. D. *Am J Physiol* **115** 94, 1936. Green, H. D., and Gregg, D. E. *ibid* **130** 126, 1940. Hall and Ichuoka<sup>-7c</sup>.

31 Kauntz, W. B., and Smith, J. R. *J Clin Investigation* **17** 147, 1938.

32 Gregg, D. E. *Am J Physiol* **114** 609, 1936. Harrison and others<sup>20</sup>. Clawson<sup>26</sup>.

33 Gross, H., and Sternberg, W. H. *Arch Int Med* **64** 249, 1939. Friedberg and Horn<sup>12</sup>.

34 Gilbert, N. C. *Quart Bull, Northwestern Univ M School* **16** 179, 1942. Gross and Sternberg<sup>33</sup>.

35 Hinrichsen, J., and Ivy, A. C. *Arch Int Med* **51** 932, 1933. Manning, G. W., Hall, G. E., and Banting, F. G. *Canad M A J* **37** 314, 1937.

36 Freedberg, A. S., Spiegl, E. D., and Riseman, J. E. F. *Am Heart J* **27** 611, 1944.

The pattern of the vessels and the changes resulting in the coronary circulation from narrowing determine the site and, to varying degrees, the intensity of the ischemia. The more pronounced other factors become, the less pronounced must be the occlusive effect of coronary arteriosclerosis in the production of anoxia with the resulting symptoms and anatomic changes.

# SUMMARY

An unselected series of 70 adult hearts were studied by the Schlesinger injection technique.

Occlusions of the coronary arteries were demonstrated in 12 of the 70 hearts. In these 12 hearts thirty-one points of obstruction were demonstrated. Thirteen were in the main stems of the three principal coronary arteries. Eighteen were in the large branches.

Interarterial anastomoses were demonstrated in all hearts with pronounced arteriosclerotic narrowing. Only in the presence of marked hypertrophy were such anastomoses demonstrated in other hearts.

In 3 of 11 hearts with old occlusions of the coronary arteries there were no old infarcts. In 2 of 5 hearts with recent occlusions of the coronary arteries there were no corresponding recent infarcts.

In 1 of 4 hearts with recent infarcts there was no recent occlusion.

Grossly recognizable scars were present in the myocardium of 26 of the 70 hearts.

Data from this work and from the literature emphasize that coronary arteriosclerosis is only one of the many factors which may be responsible for the anatomic changes and the symptoms resulting from myocardial anoxia.



## PERIARTERITIS NODOSA PRODUCING ANEURYSM OF THE RENAL ARTERY AND HYPERTENSION

PETER A. HERBUT, M.D., AND ALISON H. PRICE, M.D., PHILADELPHIA

That one form of hypertension in man is of renal origin has been accepted by most observers. From a review of the literature it is apparent that the recorded cases may be divided into two groups, namely, those in which the primary disease affected the main renal artery or its orifice thereby producing narrowing of the lumen and renal ischemia, and those in which the disease affected primarily the renal parenchyma itself. The reported causes of the former include arteriosclerosis,<sup>1</sup> periarteritis nodosa,<sup>2</sup> congenitally misplaced plug of smooth muscle,<sup>3</sup> dissecting aneurysm<sup>4</sup> and thrombosis of the main renal artery,<sup>5</sup> while the reported cases of unilateral disease of the renal parenchyma include pyelonephritis, renal calculi, hydronephrosis, infarcts, tuberculosis, cortical abscesses and hypernephroma.<sup>2a</sup>

In spite of the ever increasing number of reports on aneurysm of the renal artery<sup>6</sup> the relationship between such a lesion and renal hypertension has not been well established. In a recent review of the literature on obstructive lesions of the main renal artery, Yuile<sup>1d</sup> found that hypertension was seen in only 8 of 77 reported cases of aneurysm of this vessel and concluded that "available data provide little more than a suggestion of an occasional association between this type of lesion and hypertension." Because of the infrequency with which an etiologic relationship has been found to exist between aneurysm of the renal artery and hypertension, the role which periarteritis nodosa might play in such a combination must indeed be small. One can be certain that this disease is not one of the common causes of aneurysm of the main

renal artery or of any of its immediate branches, for these causes, as listed by Mathe,<sup>6a</sup> Howard, Suby and Harberson<sup>6b</sup> and Lowsley and Cannon,<sup>6c</sup> consist of trauma, congenital defects in the arterial wall, arteriosclerosis, syphilis and severe infections such as pneumonia, pleurisy, rheumatic fever, endocarditis, malaria, dysentery and chronic nephritis. Although in each of the three reviews just referred to periarteritis nodosa is also listed as a possible cause in none is the number of recorded cases stated nor is any reference given to a report of cases in which it was a cause. Thus Mathe<sup>6a</sup> said that "only 9 of the 56 cases reviewed had arteriosclerosis, only 2 were suffering from syphilis and an equally small number presented periarteritis nodosa." Howard, Suby and Harberson<sup>6b</sup> said the same thing in practically the same words and Lowsley and Cannon<sup>6c</sup> reported that "periarteritis nodosa although occasionally generalized displays a particular affinity for the renal artery and a small number of cases have shown this pathologic entity at autopsy."

Thus, because aneurysm of the main renal artery or its immediate branches developing on the basis of a previous periarteritis nodosa is not common, and because the association of such an aneurysm with the development of hypertension has never been satisfactorily demonstrated, we are recording 2 cases.

### REPORT OF CASES

**CASE 1**—A 44 year old Italian man, who had no noteworthy previous illnesses, consulted his physician because of gross hematuria and dull pain in the left costovertebral angle in July 1943. At the time of this and other visits to his physician his blood pressure ranged from 116 to 130 mm of mercury systolic and 68 to 74 mm diastolic. Because his symptoms were unaccounted for, he was sent to another hospital for study, where both an indigo carmine test and a retrograde pyelogram showed no abnormality in either kidney. A urinalysis disclosed albumin (a trace), erythrocytes and a few pus and epithelial cells. There were 17,600 leukocytes per cubic millimeter of blood with 80 per cent polymorphonuclear cells and 3 per cent eosinophils. The hematuria and the lumbar pain disappeared, and he was entirely well until December 1943, when severe headaches, dizziness and blurring of vision developed. With these symptoms he was admitted to the Jefferson Medical College Hospital on April 1, 1944.

Physical examination gave negative results except for a blood pressure of 170 mm of mercury systolic and 120 mm diastolic and "diffuse hypertensive retinal changes with arteriosclerosis, grade III." The eye-grounds showed both arteriolar changes in the form of perivasculitis, calcification and obliteration and, of more recent origin, edema of the nerve heads, cotton wool exudates and flame-shaped hemorrhages. Several

From the Department of Clinical Laboratories of Jefferson Medical College Hospital and the Department of Medicine of Jefferson Medical College.

1 (a) Blackman, S. S. *Bull. Johns Hopkins Hosp.* **65** 353, 1939. (b) Richardson, G. O. *J. Path. & Bact.* **55** 33, 1943. (c) Stewart, C. F. *J. A. M. A.* **114** 2099, 1940. (d) Yuile, C. L. *Am. J. M. Sc.* **207** 394, 1944.

2 (a) Abeshouse, B. S. *Surgery* **9** 942, 1941, **10** 147, 1941. (b) Spiegel, R. *Arch. Int. Med.* **58** 993, 1936. (c) Wever, G. K., and Perry, I. H. *J. A. M. A.* **104** 1390, 1935.

3 Leadbetter, W. F., and Burkland, C. E. *J. Urol.* **39** 611, 1938.

4 Riggs, T. F., and Satterthwaite, R. W. *J. Urol.* **45** 513, 1941.

5 Saphir, O., and Ballinger, J. *Arch. Int. Med.* **66** 541, 1940. Yuile<sup>1d</sup>.

6 (a) Mathe, C. P. *J. Urol.* **27** 607, 1932. (b) Howard, H. H., Suby, H. I., and Harberson, J. *ibid.* **45** 41, 1941. (c) Lowsley, O. S., and Cannon, E. M. *J. A. M. A.* **121** 1137, 1943.

urinalyses disclosed a specific gravity around 1008, traces of albumin in some specimens and a few hyaline casts and leukocytes but no erythrocytes. A phenol-sulfonphthalein test showed less than 5 per cent of the dye excreted in fifteen minutes and 10 per cent in thirty minutes. The urea clearance was 70 per cent of normal. The Wassermann and Kahn tests of the blood and the Wassermann test of the spinal fluid were negative, and the blood urea nitrogen and proteins were normal. An intravenous urogram and a retrograde pyelogram disclosed that the calices and the pelvis of each kidney were normal.

Two weeks after admission the patient suddenly became dizzy, flaccid paralysis of the left side of his body developed and he died in coma twenty-four hours later. Prior to death the blood pressure was 230 mm of mercury systolic and 110 mm diastolic.

*Necropsy* (eight and a half hours after death).—The left kidney weighed 80 Gm and measured 9 by 6 by 2 cm. Its upper third was irregularly atrophied to about one-half its normal size (fig 1). The external



Fig 1 (case 1).—Photograph of the left kidney showing ischemic atrophy of its upper portion and a saccular aneurysm of the main branch of the renal artery supplying this portion of the kidney. The vessel and the aneurysm were opened. Both are shown re-touched in the photograph.

surface was superficially and deeply scarred, and to it the capsule was densely adherent. When the latter was stripped, the surface was in addition finely granular. The lower two thirds of the left kidney was normal in shape and size. The capsule stripped easily, leaving a smooth, moist, glistening, reddish brown surface. Cut surfaces showed in the upper portion a pale brown parenchyma with a marked reduction of both the cortex and the medulla and an obscuring of the demarcations between the two. There was likewise no sharp line of delineation between the atrophic upper third of the kidney and the relatively normal lower two thirds. Cut surfaces of the lower portion of the left kidney revealed essentially normal cortex and medulla with a sharp corticomedullary demarcation. The left renal artery along with its aortic orifice and its branch to the lower portion of the kidney were normal. The main branch to the upper portion of the kidney, however, contained, 1 cm

distal to its origin, a saccular aneurysm 1.5 cm in diameter. Externally the latter was well circumscribed and was attached to the artery posteriorly by a pedicle measuring about 0.9 cm in diameter. The wall of the aneurysm was fibrotic and was 0.2 to 0.3 cm thick. The inner surface was smooth, and the lumen contained a recent soft reddish gray blood clot. The ostium between the aneurysm and the renal artery was oval and measured 0.5 cm in its greatest diameter. The inner surface of the aneurysm was uninterruptedly continuous with that of the vessel. The lumen of the latter at its junction with the ostium of the aneurysm was considerably narrowed, measuring 0.2 cm across. That just distal to the aneurysm was so small that it was found only with difficulty. It measured less than 0.1 cm in diameter. Beyond this constriction the renal vessel expanded again to an internal circumference of approximately 0.6 cm and pursued its normal course into the renal parenchyma.

The right kidney weighed 210 Gm and measured 11 by 8 by 4 cm. The capsule stripped easily, leaving a smooth, reddish brown surface. Cut surfaces showed sharp corticomedullary demarcations and no abnormalities. The larger vessels were not prominent. The entire extrarenal portion of the right renal artery, including its aortic orifice, was normal.

Along the attachment of the mesentery to the small intestine there were many shothike, round, firm nodules measuring from 0.1 to 0.5 cm in diameter. Sometimes they were partly within the wall of the intestine but usually they were within the mesentery itself, and most of them were attached to the terminal ramifications of the mesenteric artery. Cut surfaces varied from gray to brown and were for the most part solid. Except for scattered atherosclerotic plaques in the aorta and the coronary arteries, there were no other grossly detectable vascular abnormalities.

The heart weighed 400 Gm. The left ventricle measured 1.5 cm and the right 0.5 cm in thickness. There was some thickening of the papillary muscles and trabeculae of the left ventricle but no other abnormality. The lungs disclosed congestion, edema and areas of consolidation in their posterior portions.

The brain weighed 1,340 Gm. The hemispheres were symmetric, but the gyri were flat and the sulci were partially obliterated. The vessels at the base of the brain were tortuous and showed atheromatous plaques. The right frontal lobe just anterior to the head of the caudate nucleus contained a recent hemorrhage, 2.5 by 4 cm in diameter, which had extended into and completely filled the right ventricle and had pushed the ventricular system to the left. The left lateral ventricle and the third ventricle also contained blood. Throughout the midbrain and the pons there were small hemorrhages, but nowhere were aneurysms seen.

Microscopic sections of many of the circumscribed mesenteric nodules that grossly appeared to be partly within the wall of the intestine revealed fibrosed aneurysmal dilatations of the terminal ramifications of the mesenteric artery. They were located in the serosa or between the longitudinal and circular muscle layers and at the periphery often showed either longitudinal or cross sections of the vessels from which they sprang (fig 2A). The portions of the arteries directly opposite the aneurysms were as a rule normal in all respects. As the ostium of the aneurysm was approached, however, there was increasing fibrosis of the adventitia, gradual disappearance of the external elastic lamina, fibrous replacement of the media, gradual or sudden disappearance of the internal elastic lamina and a varied degree of proliferation of the subendothelial connective tissue of the intima. Sometimes this was so great as to obliterate the lumen of the ostium. Beyond this the artery was completely lost in a thick concentric mass

of dense fibrous tissue in which the coats of the vessel were no longer recognized. The lumens of the aneurysms were usually small, comprising less than one half of the entire external diameter. They were either empty or filled with well preserved erythrocytes or recently clotted blood. Throughout the areas of fibrosis there were deposits of golden brown pigment which took a positive stain for iron. These deposits were particularly abundant in the adventitia, where they were both free and within phagocytes. Occasionally the periphery also contained a few plasma cells and lymphocytes.

The upper branch of the left renal artery was cut into several blocks so as to include the junction of the artery with the aneurysm, the aneurysmal wall itself and the artery just distal to the aneurysm. The portion of the vessel directly opposite the aneurysm was essentially normal. As the latter was approached, however, the following changes were noted: increasing fibrosis of the adventitia, some attenuation of the external elastic lamina, increasing replacement of the media with fibrous tissue, in some areas preservation and in others

In all sections, throughout the areas of fibrosis but particularly prominent in the adventitia there were deposits of golden brown pigment which gave a positive reaction for iron. In these same areas there were varied numbers of plasma cells, lymphocytes and phagocytes. Calcification was not seen. There was no recognizable old or recent thrombus either within the aneurysm or within the renal artery itself.

Microscopic sections of the ischemic portion of the left kidney showed a condensation of all the elements. In the cortex there were a few scattered wedge-shaped areas of complete fibrosis, where the glomeruli were represented as round, completely hyalinized globules. At the apexes of these triangular areas, which pointed to the medulla, there were larger arteries that were partially or completely occluded by extensive subintimal proliferation of fibrous tissue without hyalinization. In some of these vessels the media was also partially or completely replaced with fibrous tissue. In all of them the adventitia and surrounding connective tissue showed marked fibrosis with a peripheral sprinkling of lympho-



Fig 2—*A* (case 1), photomicrograph of a terminal mesenteric artery as it enters an aneurysm, showing increasing adventitial and medial fibrosis, loss of the external elastic lamina, splitting and finally disappearance of the internal elastic lamina, marked subendothelial proliferation of the intima, and fluid and recently clotted blood in the lumen. Elastic tissue stain,  $\times 48$ .

*B* (case 1), photomicrograph of the aneurysm of the left renal artery at its junction with the vessel, showing fibrosis of the adventitia, some attenuation of the external elastic lamina, increasing replacement of the media with fibrous tissue, fibrillation and complete disappearance of the internal elastic lamina and severe fibrosis of the intima. Elastic tissue stain,  $\times 48$ .

*C* (case 2), photomicrograph of a section of a large artery in the hilus of the right kidney, showing diffuse infiltration with cells of inflammatory origin, granulation tissue replacement of the adventitia, almost complete destruction of the internal elastic lamina, marked thickening of the intima by early granulation tissue and a recent thrombus in the lumen. Hematoxylin and eosin,  $\times 72$ .

fibrillation, attenuation or complete disappearance of the internal elastic lamina, increasing proliferation of the subendothelial connective tissue of the intima (fig 2 *B*). These changes became increasingly severe until the fibrosed adventitia was separated from the completely fibrosed and fused media and intima by varied amounts of the external elastic lamina. This constituted the wall of the aneurysm itself. The changes in the renal artery at its exit from the aneurysm were similar to those just described with the exception that the shape of the vessel was still maintained and that subendothelial proliferation of the intima with marked encroachment on the lumen was even more conspicuous

cytes and plasma cells. Throughout the rest of this portion of the kidney, where the tissue was only condensed, the arteries showed little or no fibrosis and the arterioles showed no evidence of endarteritis obliterans. The glomeruli were normal. The tubular epithelium showed some degeneration. The interstitial connective tissue was condensed but not otherwise increased and showed no inflammation.

Histologic sections of the grossly normal portion of the left kidney and of the right kidney were similar. There were only a few scattered fibrosed glomeruli, the rest were entirely normal. The tubular epithelium showed considerable degeneration, and the interstitial

connective tissue was not increased. The larger and the smaller arteries were remarkably free of fibrosis or arteriosclerosis. Whereas some of the arterioles were normal, many showed marked endarteritis obliterans, in some instances to such a degree that the lumen was almost entirely obliterated.

Microscopic sections of the brain from the edge of the ventricular hemorrhage showed degeneration of the brain substance and scattered perivascular hemorrhages. The cerebral arteries and arterioles about 1 cm from the area of destruction showed considerable intimal and adventitial fibrosis. The periphery of the latter contained plasma cells, lymphocytes, occasional polymorphonuclear leukocytes and scattered deposits of golden brown pigment.

Sections of the rest of the organs together with portions of the pectoralis major muscle and the diaphragm failed to disclose recent or old periarteritis nodosa or other contributory changes.

**CASE 2**—A Negro man 30 years old was admitted to the Jefferson Medical College Hospital with a history of pains in the legs and the lumbar region of six days' duration and generalized pain, weakness and anorexia of four days' duration. Physical examination disclosed injection of the pharynx, enlargement of the cervical lymph nodes and extreme tenderness of the calf muscles. The temperature was 100 F, the blood pressure was 180 mm of mercury systolic and 130 mm diastolic, the blood count was normal except for a leukocyte count of 16,000 cells per cubic millimeter, and the Wasseimann and Kahn tests of the blood were negative.

During the next two weeks the pains persisted, the temperature varied from 100 to 102 F, and the blood pressure from 180 to 140 mm of mercury systolic and 130 to 100 mm diastolic. On the fifteenth day after admission the patient began to have localized pain, tenderness and muscle guarding in the right upper quadrant of the abdomen. Four days later this pain became severe, the leukocytes numbered 26,000 per cubic millimeter, and the blood pressure was 110 mm of mercury systolic and 80 mm diastolic. Within the next four days his condition rapidly deteriorated, and he died in shock twenty-three days after admission.

**Necropsy**—An extensive recent retroperitoneal hemorrhage on the right side extended from the upper pole of the right kidney to the pelvic brim. This originated in a tear of the parenchyma of the lower pole and lateral margin of the right kidney that measured 2.5 cm in length and 2.0 cm in depth. The rupture was filled with recently coagulated blood, and the surfaces were irregular, dark red and partially necrotic. In the depth of the defect there was a ruptured aneurysm, 1.5 cm in diameter, which also contained recently clotted blood. When the capsule and perirenal hemorrhage were stripped away, the right kidney weighed 370 Gm and measured 15 by 8 by 6 cm. The left kidney weighed 420 Gm and measured 16 by 8 by 5 cm. Both capsules stripped easily, leaving smooth external surfaces. Cut surfaces of each kidney showed numerous raised, brown to deep red nodules measuring 0.1 to 1.0 cm across. They were scattered throughout the kidneys but were particularly prominent at the hilum, where they were seen to be direct expansions of the main branches of the renal arteries as they entered the renal substance. The nodules were firm and on section disclosed central areas of fluid and clotted blood. The cortices and medullas were broad and the demarcations between them sharp. The extrarenal portions of the renal arteries and their aortic orifices were grossly normal.

Similar deep red nodules, often seen to be grossly related to vascular ramifications, were encountered in the omentum, the mesentery, the intestines, the pan-

creas, the gallbladder, the liver and the pericardium. In the intestines they were found in the lower part of the ileum and in the colon and in all instances were intramural. There was considerable enlargement of the abdominal lymph nodes. The heart weighed 520 Gm, and the lungs showed congestion and edema. Permission to examine the brain was not granted.

Microscopically the medium-sized and smaller arteries of the kidneys, intestines, mesentery, omentum, lymph nodes, gallbladder, liver, adrenal glands, pancreas and heart were affected with fulminating acute periarteritis nodosa. The vessels of the lungs and the spleen were not involved. In each kidney, in addition to sections made from the cortex, the smaller extrarenal branches of the renal arteries and their continuations into the hilum of the kidney were also examined and showed either patchy or complete involvement in the inflammatory process (fig 2C).

All the lesions were in the acute stage, showing no attempt at healing. The adventitia and a varying portion of the immediately surrounding tissue were densely infiltrated by polymorphonuclear leukocytes, eosinophils, plasma cells and lymphocytes. In some of the arteries, where the process was less severe, there were fewer inflammatory cells and increasing degrees of fibroblastic proliferation. The media was variously involved. In some arteries only the outer or the inner portion or both showed fibrinoid necrosis. In others the entire musculature was replaced with deep pink-staining fibrinoid material and contained inflammatory cells and nuclear fragments. In those vessels in which only the outer portion of the media and the adventitia were involved, the internal elastic lamina was often entirely normal. When, however, the inner portion of the media and the intima were involved, the internal elastic lamina was either fibrillated or, in the more severely involved arteries, completely absent. Usually the intima was necrotic and infiltrated by cells similar to those found in the adventitia, but occasionally there was considerable fibroblastic proliferation of the subendothelial connective tissue. The endothelium in the involved areas had disappeared. The lumens of the vessels were empty or filled with necrotic material and clotted blood. While many of the vessels still maintained their normal contours, some showed small and others large aneurysmal dilatations. Often the aneurysms were so large that the wall was composed of a thin rim of inflammatory tissue in which vascular structures could no longer be recognized.

In addition to the vascular changes the kidneys showed varied degrees of infiltration of the interstitial connective tissue with plasma cells, lymphocytes and eosinophils. These infiltrations appeared to follow the distribution of the vessels. Except for small focal areas of necrosis about some of the aneurysms the glomeruli and the tubules were normal. Many of the afferent arterioles were also normal, but others showed advanced sclerosis with hyaline replacement of the intima. Endarteritis obliterans, however, was not found.

#### COMMENT

In the first case the abdominal pain that occurred ten months before death was due to periarteritis nodosa of the left renal artery supplemented, perhaps, by similar involvement of the branches of the superior mesenteric artery. It was undoubtedly further aggravated when the lumen of the renal artery was occluded to such a degree that the whole upper portion of the left kidney became ischemic. That this ischemia was accompanied with true wedge-shaped infarcts

of the cortex is borne out by the areas of cortical fibrosis observed at necropsy. Either the ischemia or the infarcts could have been responsible for the hematuria. As hypertension did not develop until sometime after the episode of hematuria, it was undoubtedly produced by the renal ischemia. Death from cerebral hemorrhage is one inevitable result of hypertension even in those cases in which the cerebral vessels are not particularly weakened by a previous disease. In this case the presence in some of the cerebral arteries of intimal and adventitial fibrosis together with peripheral plasma cells, lymphocytes, occasional polymorphonuclear leukocytes and deposits of golden brown pigment is evidence that these vessels were involved in the same inflammatory process as were the branches of the superior mesenteric and left renal arteries. Whether this was enough to weaken the wall, and so predispose to a rupture, or whether a small, undiscovered aneurysm was the source of the cerebral hemorrhage remains a matter of conjecture.

Pathologically, there is little doubt that the renal aneurysm in this case was on the basis of previous periarteritis nodosa. Grossly this is supported by other evidence of periarteritis nodosa in the form of numerous aneurysms of the branches of the superior mesenteric artery—a site of predilection for this disease. Histologically, too, sections of the renal vessel and aneurysm were similar to those of the mesenteric vessels and each corresponded to the healed stage of periarteritis nodosa described by Arkin.<sup>7</sup> In each the involved portion of the vessel showed fibrous tissue replacement of the adventitia, the media and especially of the subendothelial connective tissue of the intima, with marked or complete occlusion of the lumen. The internal elastic lamina was partially or completely destroyed. Scattered deposits of iron pigment, especially in the adventitia, spoke of old hemorrhages, and the occasional presence of peripheral plasma cells and lymphocytes indicated the passing phase of the granulation tissue stage.

That the second case was one of acute periarteritis nodosa needs no further elaboration or proof. It differs from the former in that the course was fulminating, the extrarenal portions of the renal arteries were not affected, and there was no grossly demonstrable renal ischemia. Many of the main branches of both renal arteries within the hill of the kidneys, however, were affected by the process, resulting in the formation of scattered aneurysms. Similar dilatations were found in the medullas and cortices. In either or both of these locations the blood supply to the kidneys could have been impeded sufficiently to liberate a "toxic substance" responsible for the hypertension. The absence of grossly visible atrophic changes in the kidneys does not in any way detract from such an explanation, for the

arterial constriction may not have been of sufficient severity to produce such changes. In dogs Goldblatt<sup>8</sup> has demonstrated that the kidneys "may show but little change or may undergo atrophy, depending upon the severity of the constriction." Then, too, the entire course of the illness may have been too fulminating for atrophic changes to develop. The time element, however, plays no part in the development of hypertension, for in dogs<sup>8</sup> and in a few human patients, such as one described by Yuile,<sup>10</sup> hypertension developed immediately after the renal artery was occluded.

Finally, the reports on arteriolar changes in the ischemic and the nonischemic kidney are far from uniform. In dogs<sup>8</sup> the arterioles in the kidneys show no changes whereas those in other organs do. In man, Blackman<sup>11</sup> and Richardson<sup>12</sup> observed arteriosclerosis and arteriolonecrosis in both ischemic and nonischemic kidneys. Leadbetter and Burkland<sup>9</sup> and Stewart,<sup>13</sup> on the other hand, reported an absence of arteriolar changes in the affected kidney while Yuile<sup>10</sup> described not only an absence of arteriosclerosis in the ischemic kidney but also marked sclerosis of the arterioles of the uninvolved kidney. In our first case the ischemic portion of the left kidney showed no arteriolar changes while the nonischemic portion of the same kidney together with the right kidney showed advanced endarteritis obliterans but no sclerosis. In the second case, although most of the vessels showed no changes, advanced sclerosis was seen in a few scattered arterioles in both kidneys. Endarteritis obliterans, however, was not seen. In neither case was there arteriolonecrosis.

#### SUMMARY

A review of recent literature discloses that cases of hypertension of renal origin may be divided into (1) those in which renal ischemia is produced by diseases affecting primarily the renal arteries and (2) those in which the renal parenchyma itself is primarily involved.

In a case of periarteritis nodosa of the renal arteries there was an old periarteritic renal aneurysm which produced narrowing of the vascular lumen, with renal ischemia, hypertension and death from cerebral hemorrhage following. In a second case multiple acute periarteritic aneurysms of the intrarenal branches of both renal arteries produced hypertension, with death resulting from rupture of one of the aneurysms.

The sequence of events in the first case indicates that an aneurysm of an extrinsic portion of a renal artery can produce hypertension provided the lumen of the vessel is occluded to a degree great enough to produce renal ischemia.

8 Goldblatt, H. Experimental Hypertension Induced by Renal Ischemia, in Harvey Lectures, 1937-1938, Baltimore, Williams & Wilkins Company, 1938, p. 237.

## HISTOLOGIC DIAGNOSIS OF RABIES

ERNESTO HERZOG, M D

CONCEPCIÓN, CHILE

Among 75 subjects suspected of having rabies, (68 animals and 7 human beings) 52 were found actually to have the disease. A comparative study was made of the different methods of morphologic diagnosis, such as the examination for Negri bodies in Ammon's horn or the histologic examination of the ganglion nodosum of the vagus nerve, the superior cervical sympathetic ganglion, or the mesencephalon and the medulla

The method which I<sup>1</sup> found independently and published in 1941 had been described in 1900 by van Gehuchten and Nélis but had been completely forgotten after the discovery of Negri bodies. In almost 50 per cent of our 52 cases of rabies we could not find Negri bodies in Ammon's horn by Lentz's method, although the simultaneous examination of the ganglion nodo-

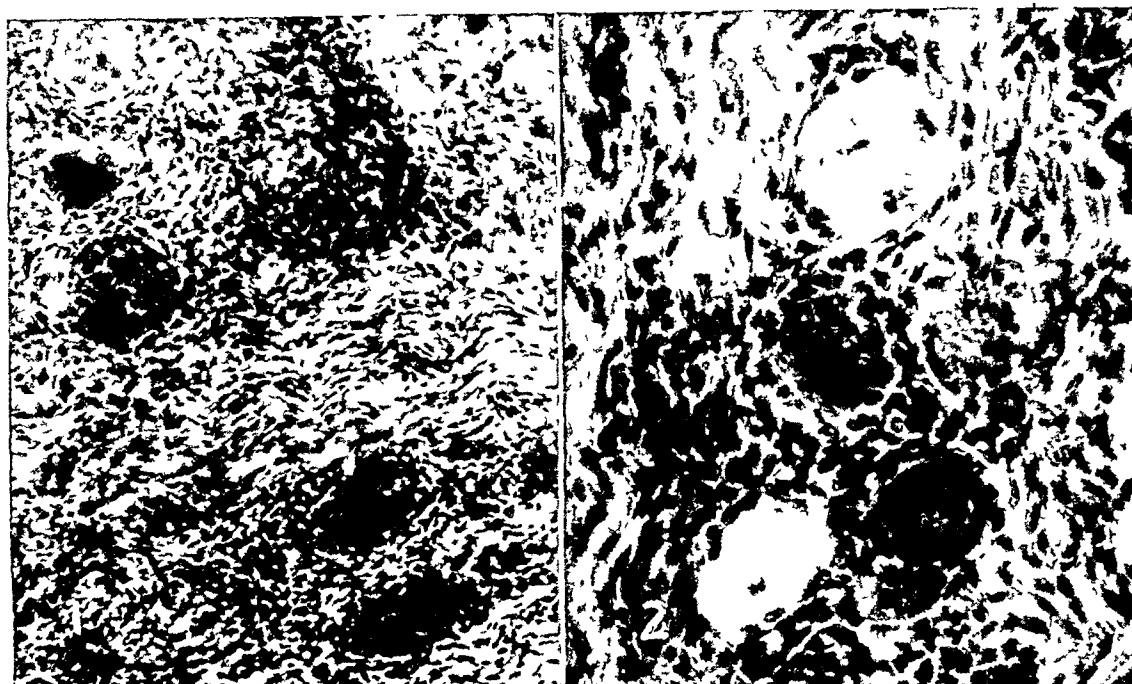


Fig 1—Diffuse rabic ganglionitis in a dog. Note two van Gehuchten nodules (residual nodules of Nageotte), with disappearance of the corresponding nerve cells, proliferation of capsular cells and inflammatory cell infiltration (neuronophagia). Nissl's method with cresyl violet,  $\times 180$ .

Fig 2—Ganglion nodosum of a dog with rabies. Note two nerve cells with chromatolysis marked by pallor and nuclear rests. Nearby is an inflammatory infiltration. Nissl's method with cresyl violet,  $\times 310$ .

The histologic examination of the ganglion nodosum always shows in cases of rabies characteristic degenerative alterations of the nerve cells, with or without inflammatory pericellular infiltrations, in focal or diffuse form, with marked neuronophagic proliferation of the capsular cells (satellites)—alterations that were constant in all of the 52 cases concerned here. Even though they are not specific they are not found in this form in the other diseases that must be considered in the differential diagnosis of rabies.

From the Institute of Pathological Anatomy, University of Concepcion, Concepción, Chile, director, Prof Dr Ernesto Herzog.

This is a summary of an article in Spanish to appear in *Revista sudamericana de morfologia* 1945, vol 3.

sum in the same cases always disclosed the alterations described in the foregoing paragraph.

The method consists of a rapid staining of frozen sections from different planes of the ganglion nodosum of each side with cresyl violet, and it permits the making of a diagnosis in less than one hour after the arrival of a suspected animal at the laboratory. If the brain is not destroyed or putrefied, a control examination of Ammon's horn, embedded in paraffin and stained by Lentz's method, a procedure which is more dependable than the taking of a smear, is added, but this delays the diagnosis eight to ten hours.

<sup>1</sup> Herzog, E. *Bol Soc. de biol Concepción*, 15 101, 1941, *Arch chilena morfol* 4 299, 1942.



A positive result of the examination of the ganglion nodosum is conclusive even if the Negri bodies are absent or cannot be sought. If the examination of the ganglion nodosum and that of Ammon's horn give negative results, histologic examination of the mesencephalon and the medulla in frozen sections may be added if the brain is in good condition, the examiner looking for the characteristic alterations of rabies encephalitis. If the results of two or three morphologic tests are negative, it is not worth while to inoculate animals, as the bitten person may be

considered free from rabies and spared the disagreeable anti-rabic injections. In doubtful cases inoculation of white mice may always be done, which gives a definite result in fifteen days.

The investigation of the ganglion nodosum has also the advantage that this structure is easily accessible even in beheaded animals. Furthermore, it is more resistant to putrefaction. In this way the examinations give a result in one or two days, even in many instances in which the result of the examination for Negri bodies is negative.

## Notes and News

**Appointments**—At the school of medicine of the University of Pittsburgh Elwyn L. Heller has been appointed assistant professor of pathology and parasitology in the place of Andrew Wallhauser, who has resigned. Dr. Heller is also pathologist to the Presbyterian Hospital of Pittsburgh.

Gilbert Dalldorf, director of laboratories of Grasslands Hospital, Valhalla, N. Y., has been appointed director of laboratories and research of the New York State Department of Health, succeeding A. B. Wadsworth, retired.

Bjarne Pearson, associate professor of pathology in Tulane University, New Orleans, has been appointed professor of pathology in the University of Vermont.

William O. Russell, formerly assistant professor of pathology in Washington University, St. Louis, is now pathologist to the Santa Barbara Cottage Hospital, visiting pathologist to the Santa Barbara General Hospital and to the St. Francis Hospital, Santa Barbara, and assistant professor of pathology in the University of Southern California, Los Angeles.

**Resignation**—Marcos Fernan-Nunez has resigned as professor and director of the department of pathology and bacteriology in the school of medicine of Marquette University, Milwaukee.

**Unveiling of Portrait**—The continuous service of Francis Carter Wood, director of the pathologic laboratories and of radiotherapy, as a member of the staff of St. Luke's Hospital, New York, for fifty years was marked by the unveiling on February 14 of his portrait painted at the direction of the board of managers of the hospital.

**Death**—John C. Grill, professor and director of the department of pathology and bacteriology in the school of medicine of Marquette University since December 1944, died on March 17 at the age of 52 years.

## Books Received

**MICROBIOLOGY AND PATHOLOGY** By Charles F. Carter, B.S., M.D., instructor in microbiology and pathology, Parkland Hospital School of Nursing and director of Carter's Clinical Laboratory, Dallas, Texas. Third edition. Pp. 777 with 225 illustrations. Price \$3.50. St. Louis: C. V. Mosby Company, 1944.

**THE MARIHUANA PROBLEM IN THE CITY OF NEW YORK** Sociological, Medical, Psychological and Pharmacological Studies. By the Mayor's Committee on Marihuana. Pp. 220. Price \$2.50. Lancaster, Pa.: Jaques Cattell Press, 1944.

**APPROVED LABORATORY TECHNIC: CLINICAL PATHOLOGICAL, BACTERIOLOGICAL, MYCOLOGICAL, VIROLOGICAL, PARASITOLOGICAL, SEROLOGICAL, BIOCHEMICAL AND HISTOLOGICAL** John A. Kolmer, M.S., M.D., Dr. P.H., Sc.D., LL.D., LL.D., F.A.C.P., professor of medicine in the school of medicine and the school of dentistry, Temple University, director of the Research Institute of Cutaneous Medicine, Philadelphia and Fred Boerner, V.M.D., associate professor of clinical bacteriology, Graduate School of Medicine, and assistant professor of bacteriology, School of Medicine, University of Pennsylvania, bacteriologist, Graduate Hospital of the University of Pennsylvania, Philadelphia. Fourth edition. Price \$10. Pp. 1,017, with 346 illustrations. New York and London: D. Appleton-Century, 1945.

**THE MARCH OF MEDICINE** The New York Academy of Medicine Lectures to the Laity, 1944. Price \$1.75. Pp. 121. New York: Columbia University Press, 1945.

**LABORATORY MANUAL FOR ELEMENTARY PHYSIOLOGY** By Lalia V. Walling, assistant professor, and Kenneth Siler, formerly instructor, University of Kansas. Fourth edition. Price \$1.50. Pp. 187, illustrated. St. Louis: The C. V. Mosby Company, 1945.

**THE NATIONAL FOUNDATION FOR INFANTILE PARALYSIS, INCORPORATED. ANNUAL REPORT, 1944** Pp. 52. New York.

**NATIONAL RESEARCH COUNCIL ORGANIZATION AND MEMBERS, 1944-1945** Pp. 88. Washington, D. C.: National Research Council, 1944.



## CHRONIC EXUDATIVE AND INDURATIVE PNEUMONIA DUE TO INHALATION OF SHELLAC

EDWIN F. HIRSCH, M.D., AND HOMER B. RUSSELL, M.D.  
CHICAGO

Purified lac, one of the natural resins known commercially as shellac, is a basic constituent of various lacquers and oil and spirit varnishes used in the industries as finishing substances. Among the solvents for the lac are methyl alcohol, butyl alcohol, amyl acetate and turpentine. Serious effects from exposure to sprays of these solvents and the dissolved ingredients may follow inhalation into the lungs. Both solute and solvent have importance. When the solvent is simple, such as methyl alcohol, its significance as a noxious agent is small, but when it is an oil, such as turpentine or linseed oil, the importance of the solvent is greater because it contains mixtures of fatty acid compounds, possibly changed by hydrolysis or oxidation at the time of inhalation or later in the tissues of the lungs. The fatty acids resulting from these chemical changes are known to have deleterious effects on animal tissues.

The composition of lac, a natural resin of insect origin, according to Bhattacharya,<sup>1</sup> depends on several factors, among them the brood of the scale insects, the plant serving as host, the climatic conditions and the method of collection. The purified product is not uniform in chemical composition. Stick lac, the resinous incrustation removed from the twigs, contains dead insect bodies, the lac dye and wax, and the true resin constituents. Most of the impurities except wax are removed in processing the shellac. Exclusive of traces of free fatty acids and dye stuff, which are difficult to remove, purified and dewaxed lac resin consists of an ether-soluble and an ether-insoluble resin. The latter is known as pure lac resin. Some evidence favors the view that the component resins of the original lac are bound together in a complex form or a weak chemical linkage. This original relationship

when disturbed during purification is not restored. The pure lac resin constituting 70 per cent of the ordinary lac, consists of hydroxy acids of the aromatic and aliphatic series joined together by lactone and various internal ester linkings. Alcohols have not been detected. Only two of the constituent acids in pure lac resin have been isolated and identified, they are (1) aleuritic, a trihydroxypalmitic acid (melting point 100 to 101 C),<sup>2</sup> forming about 30 per cent, and (2) shellolic, which, according to Harries and Nagel, is a dibasic dihydroxyhydroaromatic acid (melting point 200 to 201 C), comprising about 10 per cent. The other 50 to 60 per cent of the pure lac resin consists of unidentified acids or, according to Harries and Nagel, hydroxy acids similar to shellolic. Some unsaturated acid is present in lac, according to Bhattacharya, contained in the unidentified 50 to 60 per cent. Barnes<sup>3</sup> reported that the ether-soluble portion of the purified lac consists of polymers of aleuritic and shellolic acids of low molecular weights. The analysis of shellac by Gibson<sup>4</sup> records an acid value of 65 to 75, an ester value of 150 to 163, an iodine value of 18 to 20 and a saponification number between 225 and 231. Saponification numbers of various fractions reported by Schaeffer, Weinberger and Gardner<sup>5</sup> ranged from 147.2 to 342.4. They concluded that most of the compounds of shellac are interesters (including lactides) of the constituent polyhydroxy acids. These statements on the chemical composition of purified lac disclose that the material is a mixture of fatty acids. Some of these acids have been isolated and their structure approximated, but others have not been identified.

<sup>2</sup> Harries, G., and Nagel, W. *Chem Abstr* **16** 3070, 1922, **17** 1007, 1923.

<sup>3</sup> Barnes, C. E. *Indust & Engin Chem* **30** 449, 1938.

<sup>4</sup> Gibson, A. J. *Chem & Indust* **62** 346 1943.

<sup>5</sup> Schaeffer, B. B., Weinberger, H., and Gardner, W. H. *Indust & Engin Chem* **30** 451 1938.

From the Henry Baird Favill Laboratory of St. Luke's Hospital.

<sup>1</sup> Bhattacharya, R. *J Soc Chem Indust* **54** 82, 1935.

The reaction of tissues to lipids containing fatty acids has been described in many publications.<sup>6</sup> Among the conclusions recorded are several of significance. Chaulmoogra oil<sup>7</sup> or its mixed esters causes extensive destruction and fibrous induration of the lungs. Tissue reactions<sup>8</sup> to oils introduced into the lungs are related to the speed of hydrolysis and the amount of free acid liberated in the tissues. Animal oils produce necrosis, fibroplastic reactions and giant cells in tissues because of the rapid hydrolysis, and the scar tissue reaction caused by chaulmoogra oil is due to the high fatty acid content of that oil. Products of cod liver oil becoming insoluble in ordinary fat solvents result from oxidation changes similar to those occurring in drying oils. The specific chronic granulation tissues produced in tissues involved in tuberculosis are caused mainly by the lipids<sup>9</sup> contained in the tubercle bacilli. Lipids containing oleic acid<sup>10</sup> cause marked necrosis and fibroplastic reactions in tissues. Gerstl and Tennant<sup>11</sup> observed that fatty acids with nineteen to twenty carbons produced necrosis, but as the length of the carbon chain increased, the tissue response became a proliferative reaction with formation of giant cells. The necrotizing property of a methyl group in various portions of the carbon chain diminished as the length of the carbon chain increased.

Some information concerning the chemical reactions occurring at the interphase of fatty acids and aqueous systems containing dissolved basic compounds was obtained from the experiments of Langmuir and Schaefer<sup>12</sup> and those of Hartsuch.<sup>13</sup> Langmuir and Schaefer found that fatty acids floating on the surface of solutions of calcium and barium salts were converted to corresponding soaps, depending on the  $p_H$  of the aqueous medium. Hartsuch reported similar results with solutions of magnesium phosphate and observed an appreciable transfer of magnesium from the aqueous solution to the oleic acid when the  $p_H$  of the water medium was 7.0. An equivalent amount of hydrogen ions passed from the oil phase into the aqueous medium, tending to change the reaction to acidity. The

reaction between such fatty acid-aqueous systems, of course, is toward an equilibrium, and where the fatty acid is in excess, the  $p_H$  of the aqueous medium shifts toward maximum acidity, which for oleic acid is approximately  $p_H$  5.7. Other fatty acids in contact with aqueous mediums probably react in a similar way when in excess, producing in the aqueous medium an acidity probably approaching the hydrogen ion level of oleic acid. Local acidity developed in tissues by fatty acids would seem significant in regard to the necrosis of the tissues and the subsequent inflammatory reaction. Evidence from many sources, accordingly, supports the view that fatty acids in human and other animal tissues cause necrosis and reactive changes ranging through cellular exudation, fibroplastic response and giant cell formation.

Tradesmen working with sprays of varnish, lacquer and shellac may inhale finely divided particles of these finishing substances. Little has been published on the harmful effects of lac substances although there are reports of irritations of eye and nose among workers with shellac sprays and also of some craftsmen who stopped working because of the effects of the sprays.

#### REPORT OF A CASE

A white man aged 59 years entered St. Luke's Hospital, Chicago, May 13, 1942 in the care of Dr. Paul Holinger because of a chronic nonproductive cough, tiredness and weakness for several years, increasing dyspnea for nine months and a loss in weight of 20 pounds (9 Kg.). He owned a furniture factory and had been exposed to wood dust and varnishes for many years. Roentgen films of the chest taken six years before admission evidenced diffuse density in the base of each lung. Later he was treated for pneumonia. His dyspnea continued and became worse. Examinations at the hospital demonstrated inspiratory rales in both lungs and slight enlargement of the heart. His blood pressure was 150 mm of mercury systolic and 90 mm diastolic. A fungous infection or a tumor of the lung was considered possible. The number of leukocytes per cubic millimeter of blood ranged between 12,000 and 15,000. The findings from other examinations of the blood were within the normal range. Nothing significant was disclosed by examinations of the sputum. Roentgen films of the kidneys, the stomach, the bowel and the bones had no unusual shadows, but those of both lungs evidenced extensive infiltrations, especially of the right lung. Bronchoscopic examination demonstrated a thick tracheal and bronchial mucosa and rigid bronchial walls. The electrocardiographic tracings indicated damage of the myocardium. Analyses of the gases of the blood demonstrated interference with pulmonary aeration, and this was considered to be the cause of the dyspnea. After two weeks of discontinuous administration of oxygen, potassium iodide and mapharsen therapy, the patient's cough became much worse and productive. The fever continued, the dyspnea became severe, and death occurred on the twenty-second day after admission.

The essential portions of the anatomic diagnosis based on the postmortem examination (trunk and neck) are marked chronic indurative pneumonia of both lungs,

6 Hirsch, E. F. *Arch Path* **31** 516, 1941.

7 Read, B. E. *J Biol Chem* **62** 515, 1924. Englebreth-Holm, J. *Klin Wchnschr* **13** 1605, 1934. Frazier, C. N., and Chen, F. K. *Philippine J Sc* **42** 269, 1930.

8 Pinkerton, H. *Arch Path* **5** 380, 1928.

9 Sabin, F. R., Dean, C. A., and Forkner, C. E. *J Exper Med*, 1930, supp 3, p 1.

10 Haggerty, C. *Arch Path* **25** 24, 1938.

11 Gerstl, B., and Tennant, R. *Yale J Biol & Med* **15** 347, 1942.

12 Langmuir, I., and Schaefer, V. J. *J Am Chem Soc* **58** 284, 1936.

13 Hartsuch, P. J. *Arch Path* **25** 17, 1938.

fibrinous pleuritis on the right side, fibrous pleuritis on the left side, hyperplasia of parabronchial, cervical and biliary lymph nodes, slight chronic fibroplastic myocarditis, nephrosclerosis and cortical retention cysts of the kidneys, atherosclerosis of the aorta and of its main branches

The markedly consolidated right lung weighed 1,210 Gm, the similar left lung, 1,180 Gm. There were no adhesions on the right side between the lung and the chest, but on the visceral pleura there was a thin layer of fibrin. The lung tissues were extensively consolidated diffusely and also in nodules. Surfaces made by cutting had dilated air passages and consolidated parenchyma. These firmly consolidated lung tissues were

connective tissues, to some extent replacing the alveolar walls or leaving them as septums of hyperemic capillaries with a small amount of supporting stroma. At other levels these fibroplastic tissues were continuous into regions with alveoli whose lumens contained small masses of fibrillar connective tissues or alveolar spaces filled with granular precipitates, polymorphonuclear leukocytes and variable numbers of mononuclear phagocytes. In many places the polymorphonuclear leukocytes were in excess, and the dilated and modified alveolar spaces and bronchioles contained dense collections of these exudate cells. The lining epithelium of some bronchioles had been changed into squamous epithelium. The walls of the bronchioles were thickened by edema-



Fig 1—Photograph illustrating the consolidation of the human lungs. The pleural surface of the right lung had a thin fibrinous exudate.

gray and gray-red, with some portions of a faintly yellow cast and granular (fig 1). The pleura of the left lung had scattered torn fibrous tissue adhesions. The consolidation of the lung was like that of the right. The heart weighed 400 Gm, and in the myocardium were small regions of gray fibrous tissue. The viscera otherwise had slight or no noteworthy changes.

The histologic preparations of the lungs demonstrated extensive organizing pneumonia (fig 2). Large portions of the right lung were vascular and had moderately edematous fibrous tissue merging with the parenchyma, into whose alveolar spaces extended edematous fibrillar

tous vascular fibrous tissues or fibrous tissues with exudates of lymphocytes and plasma cells. Some alveolar spaces contained mainly fibrin, others, red blood cells, a moderate number of polymorphonuclear leukocytes and a few mononuclear phagocytes. The indurative and inflammatory changes involved most of the lung parenchyma. A few alveolar spaces had hyperemic walls, lumens with granular precipitates and a few desquamated lining cells or mononuclear phagocytes.

The tissues from the left lung were similar. Several of the large blood vessels contained dense masses of fibrin and red blood cells with leukocytes like a thrombus.

but without organization. The leukocytic exudative inflammation was especially marked in this lung and afforded little clue as to the character of the inflammatory agent excepting that in some of the aggregates of polymorphonuclear leukocytes and also in other tissues there were numerous small round vacuolated spaces

no appreciable component of the tissue reactions. The large air passages had lumens with masses of polymorphonuclear leukocytes and a small number of mononuclear phagocytes. Lung tissues fixed in solution of formaldehyde, sectioned by the freezing method and stained with sudan III demonstrated in the exudates and



Fig 2—Photomicrograph illustrating the exudative and indurative pneumonia of the human lungs,  $\times 198$ . *A*, dilated alveoli filled with masses of polymorphonuclear leukocytes and fibrin. Small vacuoles are faintly visible in the exudates. *B*, alveoli with exudates and lung parenchyma with fibroplastic tissues. *C*, lung parenchyma replaced by fibroplastic tissues with cellular exudates. Some of the fibrillar tissues have small vacuoles. *D*, alveoli filled with edematous fibrillar connective tissue, and others markedly changed and with exudate cells.

where some liquid substance had been removed in the preparation of the sections. These presumably were the places which in sections stained with sudan III contained quantities of lipid material. Giant cells were

in some of the fibroplastic tissues clusters of lipid globules and macrophages laden with lipid material.

Forty-five grams of the lung tissues fixed in solution of formaldehyde were extracted with alcohol and ether

The extract yielded a viscous brown oily residue of approximately 2 Gm weight. Titration with tenth-normal sodium hydroxide disclosed no free acid. A saponification number of 178 was obtained after hydrolysis with alcoholic potassium hydroxide. The oily residue dissolved in ethyl alcohol, ether, benzene and acetone. It dried slowly to a viscous adherent mass without a surface film like a drying oil. A sample of linseed oil was insoluble in ethyl alcohol but dissolved freely in ether, turpentine and benzene. Accordingly, the material extracted from the lungs seems to be a lac substance in an oil solvent. Alcohol and ether extraction of other portions of the lungs yielded quantities of similar material.

sections and in a solution of formaldehyde for the preparation of sections stained with sudan III.

Emulsions of the commercial shellac material in twenty-four hours caused hyperemia and multiple hemorrhages in the lungs. After seven days the lungs had scattered tan-brown nodules composed of alveolar spaces filled with exudates of polymorphonuclear leukocytes, large vacuolated macrophages and foreign body giant cells. The lumens and the walls of the bronchi showed similar exudates. Preparations stained with sudan III demonstrated globules of lipid material in the alveolar spaces and in the macrophages. The inflammatory changes were more marked after nine days, and after forty-four days the regions of consolidated lung tissues

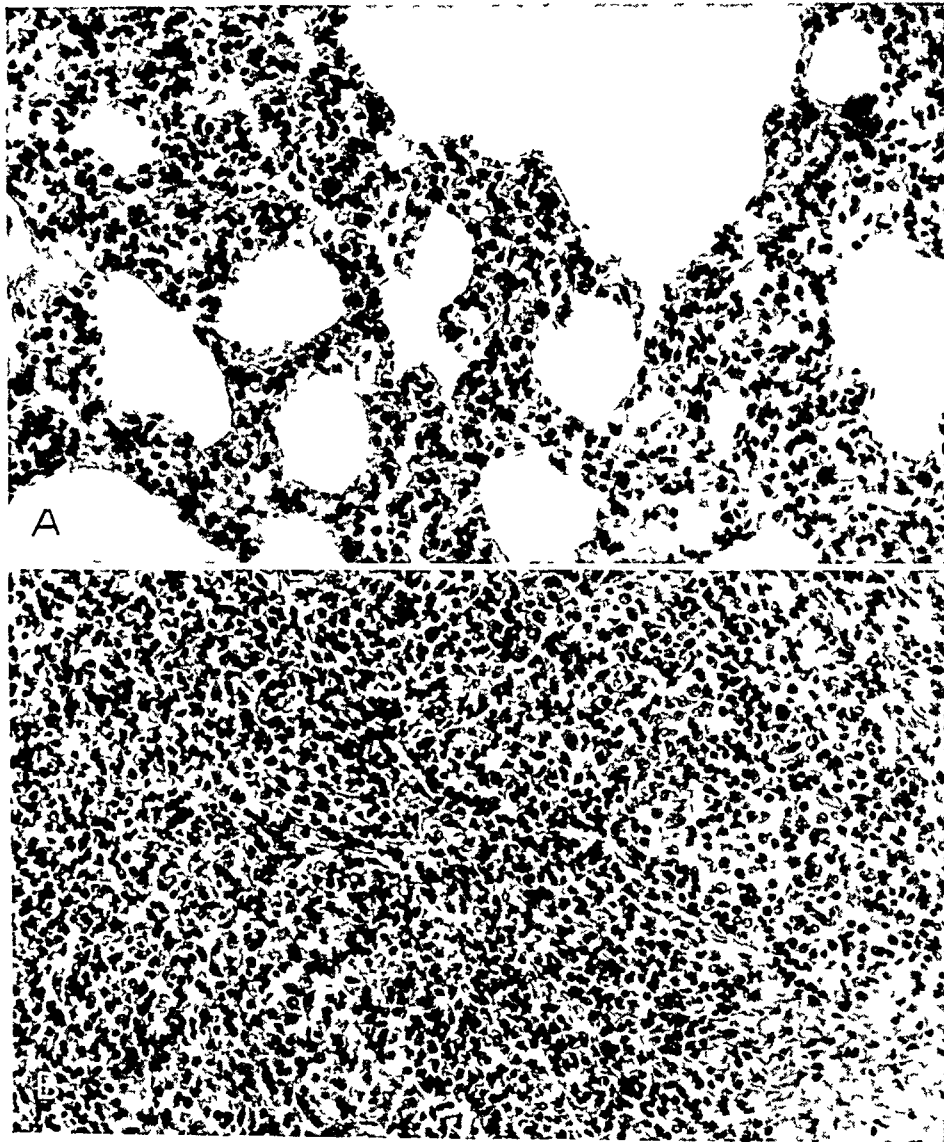


Fig 3—Photomicrograph illustrating changes in the lungs of rabbits three months after intratracheal injection of a 5 per cent emulsion in glycerin of, *A*, commercial shellac and, *B*, a laclike material recovered from the tissues of human lungs,  $\times 198$

A 5 per cent solution of the viscid residue in 95 per cent ethyl alcohol was emulsified in nine parts of glycerol. A similar emulsion was prepared with the viscid brown residue obtained by evaporation of the methyl alcohol solvent of a commercial shellac varnish. Quantities of these emulsions ranging from 0.5 to 2 cc were injected intratracheally into the lungs of rabbits. The lungs of these animals were examined for tissue changes at intervals between one and ninety days. After gross examination the lung tissues were fixed in Zenker's solution for the preparation of hematoxylin-eosin stained

had foci of necrosis, leukocytic exudate cells, fibroplastic tissues, macrophages with lipid material and foreign body giant cells (fig 3 *A*).

The tissue reactions in the lungs of rabbits receiving intratracheal injections of the emulsified viscid laclike material recovered from human lungs were much more marked. After seven and eight days there was marked hyperemia of the bronchi, and the lungs had many large firm tan-brown nodules. The histologic preparations demonstrated marked necrosis of the lung tissues. The alveolar spaces contained exudates of polymorpho-

nuclear leukocytes, fibrin, many epithelioid cells, giant cells and large vacuolated macrophages. Sections stained with sudan III showed scattered deposits of lipid material among the exudate cells and had macrophages filled with lipid particles. The lungs of 1 rabbit after three intratracheal injections at intervals of one week had similar but more extensive changes, and the lungs of another rabbit three months after a single injection had many nodules in which the alveolar spaces contained fibroplastic tissues with epithelioid cells, large vacuolated macrophages and a few foreign body giant cells (fig 3 B). The lungs of a rabbit seven days after an intratracheal injection of 1 cc of glycerol had no changes.

#### COMMENT

Bilateral chronic exudative and indurative pneumonia developed insidiously in the lungs of a man engaged in a furniture-manufacturing business. The diffuse exudative and indurative consolidation of the lungs with only minimal deposits of fibrin on the visceral pleura resembled grossly, or at least suggested a resemblance to, the lung changes with massive aspiration of liquid petrolatum. Alcohol and ether extraction of these lung tissues demonstrated the presence in large quantities of a viscous material whose general solubility and simple chemical and physical properties approximated those of a wood-finishing substance like shellac dissolved in some oil medium. Presumably this material entered the lungs by inhalation of small particles dispersed by pressure in sprays. The particles of material gradually accumulated in the terminal bronchioles and alveolar spaces of the lungs, and the irritant properties of the material caused the chronic exudative and fibroplastic inflammation. This causal relation was tested experimentally by injecting emulsions of the shellac-like material recovered from the human lungs into the tracheas of rabbits. The lungs of these animals examined later revealed extensive exudative and fibroplastic inflammation.

Chronic inflammation of the lungs caused by lac substance and certain oil solvents is another form of a pulmonary disorder commonly designated as lipid pneumonia. The changes in the lungs demonstrated by microscopic examination of the tissues differed from those with liquid petrolatum in that there was a much greater exudation of polymorphonuclear leukocytes and an abundant fibroplastic tissue response in the

alveolar spaces and bronchioles, without the presence of many large mononuclear lipophages. In these respects the reactions were more like those reported in tissues with chaulmoogra oil. The tissue reaction observed in the human lungs and those observed experimentally in the lungs of rabbits following the intratracheal injection of the extracted laclike material were due probably to the fatty acid composition of this material. Although the composition of the pure lac resin has not been established and probably varies considerably in different preparations, at least two constituent fatty acids have been isolated: (1) aleuritic, a trihydroxypalmitic acid comprising about 30 per cent and (2) shellolic acid believed by some to be a dibasic dihydroxyhydroaromatic acid comprising about 10 per cent. The remaining 50 to 60 per cent of the resin consists of fatty acids unidentified or regarded as hydroxyacids similar to shellolic. The presence of fatty acids in tissues is known to cause necrosis and marked exudative and fibroplastic tissue reactions. Some of these tissue changes seem related to the acidity developed by the migration of hydrogen ions into the aqueous tissue medium in exchange for base ions entering the oil at the water-oil interphase.

#### SUMMARY

Chronic progressive induration developed in the lungs of a man engaged in manufacturing furniture. The postmortem examination demonstrated chronic diffuse exudative and indurative pneumonia in both lungs. The character of the consolidated tissues in the lungs and the small deposits of fibrin on the visceral pleura suggested grossly the possibility of some form of lipid pneumonia. Ethyl alcohol and ether extraction of the lungs yielded large amounts of a viscid material like shellac dissolved in an oil medium. This material injected into the tracheas of rabbits caused necrosis and marked exudative and fibroplastic inflammation of the lungs. The exudative and fibroplastic tissue response approximates the changes reported in tissues with oils like chaulmoogra. Lac substances have a high content of certain fatty acids. Fatty acids of this character are known to cause necrosis and marked exudative and proliferative tissue reactions.

# DIFFUSE MENINGEAL FIBROBLASTOMA OF THE BRAIN AND SPINAL CORD

A REPORT OF THREE CASES

SAMUEL R HAYTHORN, M D, WILLIAM SHAPER, M D

AND

HUBERT C STEWART, M D

PITTSBURGH

The three cases of diffuse meningioma to be discussed in this paper are being reported because cases of this type of meningioma are rare and because they furnish additional evidence of the fibroblastic nature of meningioma whether of the diffuse or of the nodular form

Instances of meningioma are usually classified separately with respect to the form of the growth, nodular or diffuse. The nodular form is relatively common and stereotyped in appearance. It is usually single, compact, spheroidal causing a castlike depression in the soft tissues adjoining it in the brain or the cord. Occasionally, multiple growths occur, but even they are separated and sharply demarcated from each other. Microscopically, nodular meningioma has a characteristic appearance which varies within a comparatively narrow range. It is made up of coarse spindle cells, resembling epithelial cells, occurring in tight whorls or in looser alveoli as the case may be. The whorls may contain deposits of collagen about the centrally placed vessels and often contain corpora amylacea or psammoma bodies. Prior to Mallory's<sup>1</sup> work, nodular meningioma was usually diagnosed as "dural endothelioma," although many other designations had been applied,<sup>2</sup> including "cylindroma," "psammoma," "epithelial meningeal cancer" and "dural epithelioma." It was usually not considered to be of the same derivation as diffuse meningioma.

Diffuse meningioma is rare and varies widely in structure. There is little in its appearance either grossly or microscopically to identify it with the local tumor. It has been variously interpreted as a specific meningeoblastic structure, a fibroblastic tumor, lipoma, angioma and sarcoma. When pigmented it has been reported

as melanoma, and after having undergone bony metaplasia it has been called osteoma.

The name "dural endothelioma" was used because the tumor was supposed to arise from the endothelial lining of the subdural space. Mallory<sup>1</sup> demonstrated fibroglia and collagen fibers in a specimen fixed in Zenker's fluid within five minutes after its removal. He restudied the embryonic development of the meninges and concluded that the layer of mesenchymal cells which goes to form the dura separates from the arachnoid to form the subdural space and that the subdural space is therefore lined on both sides by a special type of fibroblast and not by endothelium. He traced the development of the whorled tumor to endothelium-like proliferations of the arachnoid villi and identified the cells that composed the whorls as fibroblasts from their ability to form fibroglia fibrils, collagen and in some instances elastic tissue. He suggested the name "arachnoid fibroblastoma" for the tumor.

Penfield<sup>3</sup> agreed with Mallory's interpretation of dural endothelioma and asserted that the fibrils in this tumor are not neuroglial but are a particular form of collagen. He changed the name to "meningeal fibroblastoma." Penfield stated that he was able to stain fibroglia fibrils with more or less difficulty in a series of meningeal tumors which he had studied.

Globus<sup>2b</sup> took exception to Mallory's identification of the tumor by means of fibrils. He reviewed the development of the meninges in fish and fowls and developed an elaborate classification for all meningeal tumors on a "phylogenetic and ontogenetic" basis. He studied 103 tumors originating in the meninges and concluded that the variation in the tumors was so great that no single type cell could be responsible. He quoted the studies on the formation of the meninges by Hallerstein, Sterzi and Gelderen to support his own views and applied them to the explanation of the diffuse meningiomatous growth. He pointed out that remnants of

From the William H. Singer Memorial Research Laboratory of the Allegheny General Hospital.

<sup>1</sup> Mallory, F. B. J. M. Research **41**: 349, 1920.

<sup>2</sup> (a) Cushing, H., and Eisenhardt, L. Meningiomas, Springfield, Ill., Charles C. Thomas, Publisher, 1938, chap. 1, p. 506. (b) Globus, J. H. Arch. Neurol. & Psychiat. **38**: 667, 1937.

<sup>3</sup> Penfield, W. Surg., Gynec. & Obst. **45**: 178, 1927.



undifferentiated meninges often persist beyond embryonic life and that these mesenchymal remains are often found in other pathologic conditions, such as hydrocephalus

Weinberger<sup>4</sup> collected all of the instances of the diffuse type of meningeal tumor that he was able to find and reported a very unusual case of his own. In Weinberger's case, in which the patient was an 8½ year old girl, the greater part of the surface of the frontal lobe was covered with a thin pinkish layer of tumor resembling the "icing on a cake." The dura was free from the tumor. The patient made a partial recovery after removal of the neoplasm and returned to school. In his discussion, Weinberger considered that the diffuse tumor represents a zonal rest of endomeninx and may form pia-arachnoid or pia alone. He postulated that the tumor is limited in extent to the original malformation because he failed to find evidence of eccentric growth, permeation through the meningeal layers or implantation by seeding. He expressed the belief that the tumor belongs with meningioma but preferred the term "meningiomatosis." Weinberger accepted the view of Globus that there is no parent cell theory which is acceptable for the origin of meningioma and that it is best accounted for on an embryologic basis. Other cases cited by Weinberger included those of Harbitz,<sup>5</sup> Arlt,<sup>6</sup> Bailey,<sup>7</sup> Bailey and Bucy,<sup>8</sup> Lichtenstein and Ettelson,<sup>9</sup> Casper<sup>10</sup> and Conner and Cushing.<sup>11</sup> Of this group the case of Arlt<sup>6</sup> was similar to our first case. A diffuse meningioma encased the spinal cord. In Harbitz's<sup>5</sup> case several large yellowish incrustations filled the sulci. The largest was in the region of the sylvian fissure and spread out over the temporal and parietal lobes and the island of Reil. The tumor was composed of loose spindle cells, and the network stained positively for collagen. Bailey's case<sup>7</sup> was reported as one of primary diffuse sarcomatosis in a 3 year old child. The neoplasm was located in the region of the cerebellum and the brain stem. In the case of Bailey and Bucy<sup>8</sup> the diffuse meningioma

occurred in a 45 year old woman and covered the leptomeninges "practically everywhere," filling the sulci and following the vessels for a short distance into the cortex. Lichtenstein and Ettelson<sup>9</sup> reported a case in which the leptomeninges of an infant were thick and white with nodules the size of a pinhead along the vessels. Casper<sup>10</sup> reported 2 cases. In one there were several nodular masses over the occipital lobes and at the base of the brain, in the other a sellar meningioma was observed, with thickening of the arachnoid over the entire brain. In the case of Conner and Cushing<sup>11</sup> there were diffuse meningeal tumors which were not visible in the gross inspection. Microscopically the condition was true sarcomatosis (endotheliomatosis), the origin was multiple and affected the adventitial sheaths of the cortical vessels. It was located in the region of the flocculus. Brown and Kernohan<sup>12</sup> reported a case in which the tumor was located similarly to that in our first case. It formed a sheath surrounding the spinal cord from the first thoracic vertebra to the conus medullaris and encased the cauda equina. They called the condition meningiomatosis rather than meningioma. Cases of pigmented meningioma have been recorded by Ray and Foot<sup>13</sup> and Brown,<sup>14</sup> and the origin of the pigmented tumor has been discussed by Taft.<sup>15</sup> A lipoblastic form has been reported by Haverfield and Walker.<sup>16</sup> Russell and Sachs<sup>17</sup> collected 4 cases of meningeal sarcoma that metastasized to distant organs and added 4 cases of sarcoma, in 3 of which there was distant metastasis. Turner and Craig<sup>18</sup> reported a case of osteogenic sarcoma of meningeal origin. They explained the preserved bone as an example of heteroplasia. Turner, Craig and Kernohan<sup>19</sup> analyzed 370 cases of intracranial meningeal tumor and considered the tumor to be malignant in 10 per cent of them. They used the term "malignant meningioma" in preference to "meningeal sarcoma" because the latter name indicates a specific tumor with an essential origin.

- 4 Weinberger, L. M. *Am J Cancer* **38** 1, 1940
- 5 Harbitz, H. *Acta path et microbiol Scandinav* **12** 24, 1935, cited by Weinberger<sup>4</sup>
- 6 Arlt, H. *Ztschr f d ges Neurol u Psychiat* **156** 713, 1936, cited by Weinberger<sup>4</sup>
- 7 Bailey, P. *Arch Surg* **18** 1359, 1929
- 8 Bailey, P., and Bucy, P. C. *Am J Cancer* **15** 15, 1937
- 9 Lichtenstein, B. W., and Ettelson, A. *Arch Path* **24** 497, 1937
- 10 Casper, J. *Deutsche Ztschr f Nervenhe* **96** 85, 1927, cited by Weinberger<sup>4</sup>
- 11 Conner, C. L., and Cushing, H. *Arch Path* **3** 374, 1927

- 12 Brown, M. H., and Kernohan, J. W. *Arch Path* **32** 651, 1941
- 13 Ray, B. S., and Foot, N. C. *Arch Neurol & Psychiat* **44** 144, 1940
- 14 Brown, H. *Arch Neurol & Psychiat* **47** 271, 1942
- 15 Taft, A. E. *Arch Path* **30** 1073, 1940
- 16 Haverfield, W. T., and Walker, A. E. *Arch Surg* **42** 371, 1941
- 17 Russell, W. O., and Sachs, E. *Arch Path* **34** 241, 1942
- 18 Turner, O. A., and Craig, W. M. *Arch Path* **32** 103, 1941
- 19 Turner, O. A., Craig, W. M., and Kernohan, J. W. *Surgery* **11** 81, 1942

## REPORT OF CASES

The first 2 of our cases have been mentioned previously<sup>20</sup> but have not been described in detail. The third case<sup>21</sup> was erroneously reported from this laboratory in 1926 as one of secondary gliomatosis of the meninges accompanying a large gliosarcoma of the brain. A recent restudy shows definitely that the meningeal tumor is a separate and distinct growth which contains nodular areas presenting a structure characteristic of the local whorled meningioma and diffuse areas of the fibroblastic type which merge gradually into each other.

**CASE 1**—The patient was a white man aged 57. His occupation was that of a railroad signalman, at which job he worked until three days before his admission to the hospital. The mother died of carcinoma of the bladder, the father, of Bright's disease. The patient had had typhoid fever and gonorrhea and had lost an eye in the first world war.

He first consulted his physician twenty-two days before his death on account of numbness in the two small toes of each foot and pain between the shoulders. The sensation of numbness began in the toes and extended upward to the buttocks and sometimes to the waistline. For four days intermittent pains had been radiating to the occipital region, and these were followed by nausea and vomiting. The vomiting was projectile and relieved the pain. Sleep was disturbed by headache. The patient felt weak and had lost 10 pounds (4.5 Kg). Six years before, he had been given a spinal adjustment for pain in the left hip. He attributed the pain in his shoulder to the severity of the manipulation.

He was fairly well developed and nourished. There were no noteworthy changes on physical examination except missed heart beats, varying from three to five per minute. The blood pressure was 130 systolic and 80 diastolic.

The Romberg test was questionably positive, with swaying to the right. There were tremors of the fingers and lips. Ataxic movements were not perceptible. The right optic disk was slightly blurred, with no elevation. The retinal vessels were not dilated. The left eye was artificial. The cranial nerves otherwise were undisturbed. The deep reflexes in the right arm and the right leg were slightly increased, with clonic movements of the right ankle jerk. The abdominal and cremasteric reflexes were absent. The plantar response was normal. Sensations of touch and pain were diminished in the saddle area of the left foot and the outer part of the right foot. Fibrillations were apparent about the deltoid muscles. There was slight atrophy in the legs.

The blood showed 5,050,000 red cells and 9,350 white cells, with neutrophils 75 per cent and lymphocytes 25 per cent. The hemoglobin was 94 per cent. The blood smear disclosed no abnormalities. The urine revealed a faint trace of albumin with hyaline casts. Bence Jones protein was not found. Chemically the blood was practically normal. The Wassermann and Kahn tests of the blood were negative. Lumbar puncture revealed clear yellowish fluid on four different occa-

sions with pressure varying from 300 to 800 mm of spinal fluid pressure. The cell count varied between 6 and 10 white cells per cubic millimeter. The total protein varied between 670 and 1,700 mg per hundred cubic centimeters. The Wassermann test of the spinal fluid was negative. The colloidal gold curve was 0000000000. On roentgen examination the skull presented no abnormality. There was a slight narrowing of the anterior part of the body of the seventh dorsal vertebra with some irregularity of its anterior surface. There was a slight degree of hypertrophic lipping of the articular edges of the fourth lumbar vertebra. Electrocardiographic tracings disclosed numerous ectopic ventricular contractions and was interpreted as sinus arrhythmia.

During his stay in the hospital the patient became progressively weaker and revealed many bizarre neurologic symptoms. For several days there was marked diminution of pain sensation up to the fifth dorsal dermatome anteriorly and the third dorsal dermatome posteriorly. Several days after admission he was unable to void and required catheterization. Gradually movements of the legs became difficult, following which the deep reflexes decreased and finally were absent.

Twelve days after admission he had a period of amnesia, followed by confusion. The neck became stiff, and there was flaccid paralysis of both legs. Slight weakness of the lower left side of the face was present, the tongue deviated to the left. Sensory discrimination of pain revealed a level at the first dorsal segment of the cord at this time, and the following day a level at the tenth dorsal segment was noted. There was also diminution of the sense of heat in the left arm, the left leg and the left side of the face. Position and vibratory sensations were lost in the feet. On this day lumbar puncture revealed a clear yellow fluid with a pressure of 800 mm. At times pain sensation was felt at the first lumbar segment.

The temperature ranged between 97.8 F and 98.4 F for twelve days, occasionally rising in the next five days to 100 F. The last two days it rose to 105 F. The respirations were 20, occasionally increasing to 22 until the eighteenth day after admission, when they rose to 40 per minute. The pulse was slow, varying between 44 and 62 until a few days before death, when it became quite rapid. He died on the nineteenth day after admission.

The clinical diagnoses considered were (1) diffuse cerebrospinal neuropathy, (2) tumor of the spinal cord, (3) injury of the spinal cord and (4) encephalomyelitis of virus origin.

**Autopsy**—The gross changes outside the brain and the cord were unimportant. The brain weighed 1,375 Gm. The dura appeared normal. The pia was congested. The brain was soft, moist and without hemorrhage or evidence of embolism or infarction. After fixation, the pia and the arachnoid appeared thickened and grayish yellow over the pons and the medulla, with extensions of this change to the under surface of the cerebellum. The grayish membrane followed the meningeal folds into the fourth ventricle and spread over the choroid plexus, where it almost completely filled the space. On cross sections the tumor was observed to surround the infundibulum of the pituitary gland and to form a thin coat about the epiphysis. It did not appear in either of the lateral ventricles. As it reached the pons it spread out in a thin layer which converged to include the medulla and the cord which it completely surrounded. Below the medulla it consisted of a membrane of variable thickness which

20 Haythorn, S. R., Shapera, W., and Stewart, H. C. *Am J Path* 18: 739, 1942.

21 Brannon, D. *Am J Path* 2: 123, 1926.

encased the cord throughout its length and covered it continuously like a stocking. In the region of the cauda it divided into fibrillary branches and followed the sheaths of the nerves. The amount of distortion and compression of the cord at any level varied with the thickness of the tumor.

*Microscopic Description*—The extent of the tumor was confirmed microscopically. A section taken from the region of the infundibulum marked the superior point at which tumor tissue was found. Another section, which included the medulla, adjoining portions of the cerebellum of each side and the choroid plexus of the fourth ventricle showed extensive neoplastic involvement. The meninges about the medulla were greatly thickened and replaced with tumor cells. The connective tissue of the velum interpositum was infiltrated with tumor cells, which grew in all directions and invaded the strands of that membrane. Tumor tissue displaced and compressed the choroid plexus and the connective tissue supporting its vascular portions.

The cervical cord was greatly distorted by a growth of the meninges which completely surrounded it and extended along the sheaths of the spinal nerves at their roots.

The tumor was made up of diffusely spread small spindle and round cells which had little cytoplasm. Many of the nuclei were pyknotic, although actual mitotic figures were comparatively rare. The tumor cells were generally arranged in nests or alveoli separated by a network of small vessels with lumens about the size of capillaries and walls that were thickened and hyalinized. The cells were arranged on a fine reticulum. The separating trabeculae were made up of collagen and there were bands of collagen about vessels. While the tumor in general replaced the arachnoid layer, there were areas in which it was separated from the white columns by the collagenous pia. In other places the pia was raised from the cord and had a layer of tumor beneath it. In still other places the pia-arachnoid was entirely replaced by tumor, so that the layers were not discernible.

Not only was the spinal cord compressed and distorted by the tumor but there were places in which the tumor actually extended into the gray matter and separated the medullary sheaths. The median raphe of the cord was completely infiltrated by it. There were degenerated areas in the lateral columns in which the myelin had disappeared and the neuraxons were swollen to several times their ordinary diameters. The ganglion cells appeared degenerated, and many of them were without nuclei.

Sections from various levels of the cord showed considerable variation in the thickness of the tumor enveloping the cord. In parts of the thoracic region the tumor was thicker than the cord itself.

Numerous stains were applied to demonstrate fibroglia fibrils, collagen, reticulum, myelin and fat. There were no fat cells in the growth. Myelin was not present except about the nerve trunks which passed through the growth. The tumor produced fibroglia, collagen and a fine argentophil reticulum which proved its fibroblastic derivation. Since it grew rapidly and invaded other tissues, it was considered sarcomatous.

The anterior lobe of the pituitary gland was compressed and smaller than usual. A part of the posterior lobe was invaded by direct extension of the tumor growth.

The pathologic diagnosis was rapidly growing diffuse meningeal fibroblastoma or meningeal sarcoma.

**CASE 2**—The patient was a white man aged 31, married, a lithographer. He was a moderate user of tobacco, alcoholic liquors and coffee. His chief complaint was headache, which began two weeks before admission, at the time a cold was contracted, and persisted until death. On the morning of admission he awoke with severe frontal headache, which later spread toward the occiput.

He was a well developed, well nourished man, lying in bed in a semistuporous condition. He was able to answer questions but raised his eyelids with difficulty. The general physical examination gave negative results. The blood pressure was 126 systolic and 70 diastolic.

There was no nuchal rigidity. There were questionable Kernig signs bilaterally. The deep reflexes were equally present. The abdominal reflexes were decreased on the right side, and the Babinski reflex was equivocal bilaterally. Ophthalmoscopic examination revealed pale and somewhat blurred disks. The cranial nerves were otherwise undisturbed. Sensations were intact. There was no motor weakness.

The patient's headache increased and he became drowsy. He vomited brown fluid. Spinal puncture showed a fluid pressure of 150 mm. All specimens were tinged with blood. The following day the blood pressure increased to 134 systolic and 80 diastolic. The pulse was 56. The patient became delirious, and ptosis of the left upper eyelid and twitching of the left arm developed. The optic disks became elevated. The general condition became gradually worse and death occurred three and a half hours after the development of the twitching of the arm.

The clinical diagnosis was spontaneous subarachnoid hemorrhage.

*Autopsy*—The brain weighed 1,400 Gm. The meninges were much congested. As the frontal lobes were lifted from the floor of the skull, a huge mass of clotted blood, which weighed 150 Gm, was found beneath the left frontal lobe. The clot was adherent to the dura over the supraorbital plate and was stripped from the under surface of the left frontal lobe with little difficulty. The under surface in the region of the second and third inferior gyri was softened to a mushy consistency, and the convolutions were compressed. The floor of the anterior horn of the left lateral ventricle was very thin and at one point measured 0.5 cm in thickness. After the brain was hardened, a zone of encephalomalacia was found which involved the inferior frontal gyrus. At the apex of the softened area was a small vessel filled with clot. The brain tissue about it contained small hemorrhages and presented a honeycomb appearance. This zone was in close relation to the clot which filled the arachnoid space. Cross sections were made at centimeter intervals and the softened area found to extend backward to a point just beneath the floor of the lateral ventricle. The clot contained whitish strands, but tumor was not suspected in the gross examination.

*Microscopic Description*—Sections were taken from the dura and the arachnoid over the supraorbital space, beneath the hemorrhage described in the gross specimen. The arachnoid was thrown into numerous folds, attached to which was a rapidly growing malignant tumor. Parts of the tumor had undergone necrosis and were infused with blood from the hemorrhagic zones. In the perivascular zones the tumor was very cellular and was made up of round, oval and spindle-shaped cells with large vacuolated nuclei and prominent nucleoli. In places the cell polarity was lost. Many cells were pyknotic, and many of them showed mitotic figures. Special stains were made of this section. The phosphotungstic acid-hematoxylin stain failed to show the presence of fibroglia or neuroglia fibrils, possibly because

the mass was fixed in a solution of formaldehyde. Masson's stain showed that portions of the growth were permeated by strands of collagen which appeared to be continuous with the collagen about the vessels and dura. Silver stains showed fine reticulum permeating the entire tumor.

Some of the sections taken from what appeared to be clot in the gross specimen showed that it was made up of both tumor cells and blood clot. The cells in these sections were multinucleated and showed many mitotic

from the brain showed thrombosed vessels with a wedge-shaped hemorrhage involving the white matter beneath the meninges. The pia-arachnoid appeared to be broken at one point. Two large sections showed the presence of thrombi in the cerebral vessels with diffuse bleeding into the meninges and into the adjoining brain tissue. In the neighborhood of the hemorrhagic areas the brain showed zones of recent encephalomalacia. One of the veins in the meninges immediately beneath the softened area showed active phlebitis with permeation of its wall

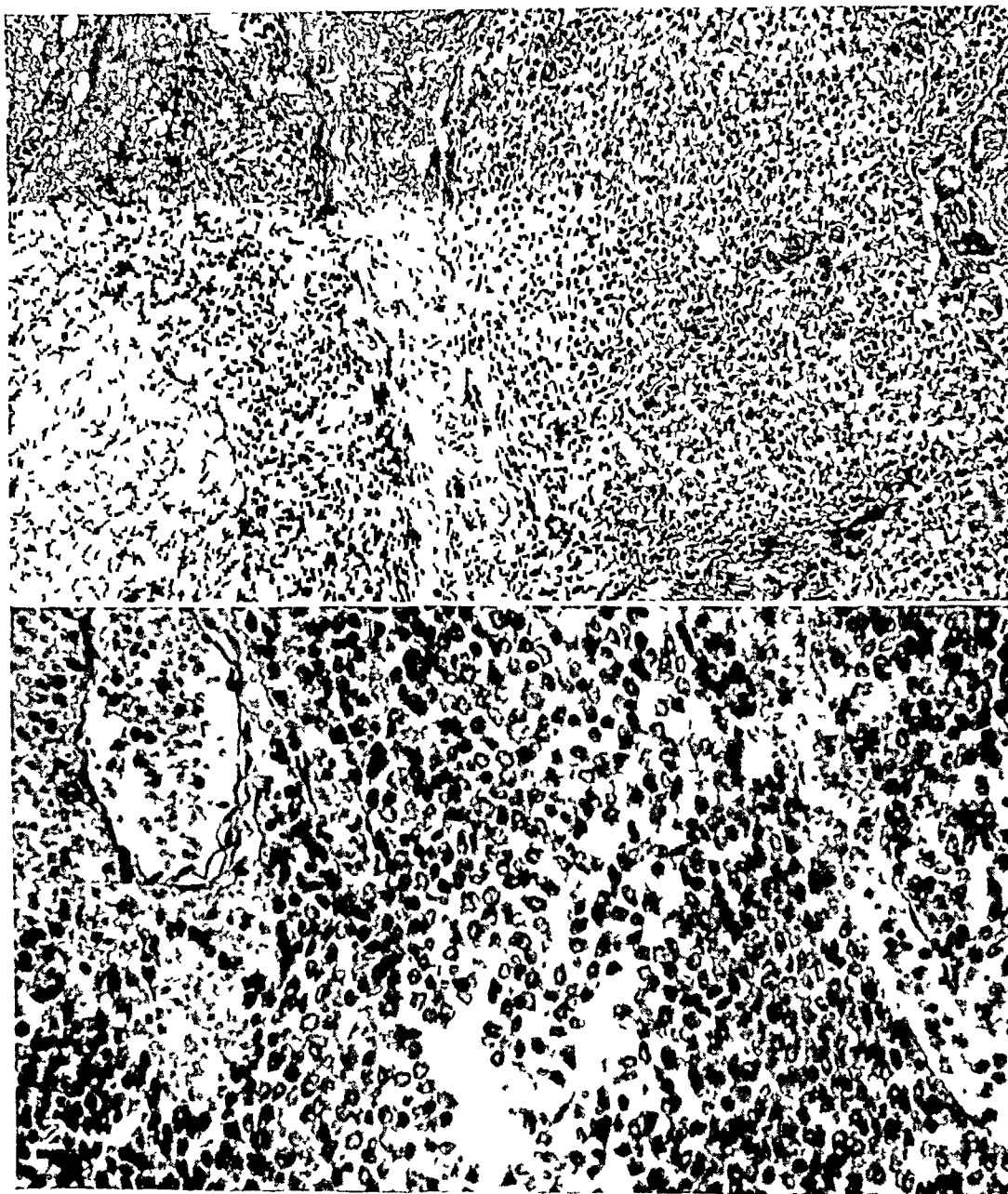


Fig 1 (case 1)—Meningeal sarcoma of the spinal cord,  $\times 90$ . At the extreme left some of the white matter of the lateral columns is seen. Adjoining the cord on the right is a portion of the growth beneath the pia. The wide collagenous band crossing from above downward is pia. At the right of it the diffuse tumor is again seen.

Fig 2 (case 2)—Meningeal sarcoma,  $\times 280$ . The photomicrograph shows the cellular nature of the growth, the absence of stroma and the thin-walled vessels.

figures and much pyknosis. There were large zones in which the cells were spindle shaped and resembled the structure of a spindle cell sarcoma.

Several sections were taken from the region of the first and second frontal gyri immediately above the hemorrhage. Where the meninges remained intact, there was an extensive subpial hemorrhage with areas of softening involving the white matter.

The brain was edematous, and the Virchow-Robin spaces were widely distended with fluid. Two sections

by segmented leukocytes and mononuclears. The cerebellum appeared normal.

The pathologic diagnosis was meningeal sarcoma with large subdural clot and localized encephalomalacia.

The third case, already described in the literature under the title of "Secondary Glommatosis of the Meninges,"<sup>21</sup> largely because it occurred in the same brain with another tumor, has been

restudied, and we are convinced that the meningeal part of the tumor is meningioma and is worthy of reconsideration because it indicates that the division of meningioma into diffuse and nodular types is a superficial one. In this case

CASE 3—A white girl 13 years old died following a craniotomy for relief of pressure. The complete history may be found in the published report.<sup>21</sup>

*Autopsy*—The weight of the brain was 1,115 Gm. Briefly, there was a 'large, firm tumor which projected from the left basal ganglia, well to the right of the mid-



Fig 3 (case 3)—Meningeal fibroblastoma of the pia-arachnoid,  $\times 90$ . The growth involved the pia-arachnoid and was not found beneath the pia. The portion of the tumor shown in this photomicrograph was made up of fibroblastic elements arranged in a diffuse and loosely woven manner, and there was no evidence of rapid growth.

Fig 4 (case 3)—Meningeal fibroblastoma of the pia-arachnoid,  $\times 90$ . The section was taken from another portion of the same tumor as that shown in figure 3. In this section the tumor is growing in the compact nodular form with quite definite whorls.

both diffuse and nodular forms were present together, and one form shaded gradually into the other. The type cell was the same for both

line and displaced the right basal ganglia." It involved the upper portions of the optic thalamus and was 4 to 5 cm long and 3 cm wide.

The meninges at the base of the brain resembled the exudate of chronic meningitis. The leptomeninges were dull and opaque. The subarachnoid space and the cisterna were filled with what was taken to be a thick exudate but which proved on microscopic examination to be tumor. The material obscured the basal vessels, the optic chiasm and the exits of the cranial nerves. It extended over the lateral and anterior aspects of the temporal lobes, the base of the cerebellum, the pons and the medulla.

Microscopically, the tumor of the basal ganglions consisted of the main mass, which was diagnosed as gliosarcoma, and a smaller nodule lying within it that was not diagnosed. Glial fibrils were not observed in either of the brain tumors with Mallory's phosphotungstic acid-hematoxylin stain. The larger mass invaded the choroid plexus, where there was no question of its secondary nature.

The growth of the pia-arachnoid over the brain stem and basilar regions, which was believed to be meningitis in the gross inspection, was made up of spindle cells arranged in the form of a diffuse membrane. It did not invade the brain from which it was excluded by the pia, but formed a fibrillar network in the arachnoid. The fibrillar network was taken to be a form of gliosis extending from the gliosarcoma although no point of connection between the tumor of the brain and the arachnoid could be found. The report states that in some portions it "somewhat resembled an endothelioma of the meninges." Phosphotungstic acid-hematoxylin staining of the meningeal tumor showed beautiful fibrils, believed at the time of the report to be glial, and coarse brown fibers suggestive of collagen.

In the restudy of the case there is nothing to add to the report itself, but in the light of the newer classification of tumors of the brain by Bailey and Cushing<sup>22</sup> and by others we have changed the diagnosis and now interpret the case as one of two unrelated intracranial tumors. We think that the large tumor of the brain would now be called "glioblastoma multiforme" and the smaller inclusion within it an isolated nodule of bipolar spongioblasts. We feel sure that the meningeal tumor is meningeal fibroblastoma and has both diffuse fibrillar and compact nodular portions.

Our reasons for these conclusions are: 1 The tumor of the brain and the meningeal tumor were cytologically different. 2 Where the tumor of the brain invaded the ependyma and the choroid plexus, its original structure was repeated and typical glial giant cells were formed. The meningeal tumor did not repeat the structure of the principal growth and did not contain giant cells. 3 The meningeal tumor was confined to the pia-arachnoid, and there was no connection between the two tumors. 4 Fibrils were not shown in the tumor of the brain but were abundant in the meningioma. While neuroglia and fibroglia fibrils stain alike with Mallory's phos-

photungstic acid-hematoxylin stain, the arrangement in this case was that of fibroglia.<sup>5</sup> There are several instances in the literature of tumor of the brain and unrelated meningioma having occurred in the same case (Cushing and Eisenhardt<sup>2a</sup>, Courville<sup>23</sup>). Courville reviewed and tabulated 134 cases of multiple intracranial tumors and stated that almost every combination of multiple intracranial tumors occurs from the standpoint of location, tissue, origin and degree of malignancy.

#### SUMMARY AND COMMENT

In 2 of the 3 cases of diffuse meningioma reported the neoplasm was invasive and showed other evidences of rapid growth, such as multiple mitoses. In the third case the growth was both diffuse and nodular. In all 3 cases the tumor involved the pia-arachnoid. In the first case it grew on both sides of the pia, and in the second it was attached to the dura and separated from the brain by the pia. In the first and third cases the tumor showed fibroglia fibrils with Zenker's fixation and Mallory's phosphotungstic acid-hematoxylin stain. In the second the tumor was fixed in a solution of formaldehyde, which does not preserve fibroglia fibrils. The demonstration of fibroglia fibrils and collagen fibers identified the tumor cell as a fibroblast. Fibroblasts are polypotential cells, capable of forming diffuse membranes, nodular structures, chondromucin, cartilage and bone, and may take up fat. Special types of connective tissue cells of the meninges may be pigmented (Taft<sup>15</sup>). The rapid course in the first case argues in favor of tumor (meningioma) instead of an embryonic mesenchymal rest as postulated by Globus<sup>2b</sup>. In all 3 cases the growth appeared to be a true neoplasm of fibroblastic origin and in our opinion should be classed as meningeal fibroblastoma rather than "meningiomas." Apparently meningiomas would be hyperplasia of meningeal elements, although we were unable to find the word in either of the most recent editions of two standard medical dictionaries.

In the first 2 cases the tumor was a fibroblastic cancerous growth, and we believe it should be called either "meningeal fibroblastoma" or "meningeal sarcoma," since substituting the term "malignant meningioma" makes a distinction without a difference. Finally, the third case ties up diffuse and nodular meningioma as variations of one and the same cytologic entity.

<sup>22</sup> Bailey, P., and Cushing, H. *Tumors of the Glioma Group*, Philadelphia, J. B. Lippincott Company, 1926.

<sup>23</sup> Courville, C. B. *Am J Cancer* 26: 703, 1936.



# MORPHOLOGIC STUDIES OF RATS DEPRIVED OF ESSENTIAL AMINO ACIDS I PHENYLALANINE

MARK E MAUN, MD

WILLIAM M CAHILL, PH D

AND

RUTH M DAVIS, BA\*

DETROIT

It is widely known that certain amino acids are necessary for growth and the maintenance of health,<sup>1</sup> but there are few studies pertaining to the effects of specific amino acid deficiencies on particular organs or tissues. Numerous investigations have shown that animals fail to grow normally if placed on diets deficient in essential amino acids. It has also been shown that in both human and animal populations fertility decreases following periods of poor nutrition<sup>2</sup>, when rats were placed on diets deficient only in tryptophan,<sup>3</sup> the animals failed to respond.

Indirect evidence of the anatomic alterations associated with deficiencies in amino acids has been presented by studies on choline metabolism.<sup>4</sup> In rats consuming low choline diets the liver became fatty and necrosis of the tubular epithelium of the kidneys developed, these changes were augmented by the addition of cystine and were alleviated by the addition of methionine. Recently more complete anatomic studies have been reported by Harris and his co-workers,<sup>5</sup> who placed rats on synthetic diets almost completely free of lysine. In the six weeks of the experimental period the animals on the deficient diets ceased to grow while those of the control group developed normally. Post-mortem examination of the animals of the deficient group showed a general wasting of soft tissue, while blood studies revealed hypopro-

teinemia and a decrease in hemoglobin as well as in red blood cells. Roentgenograms of bones showed decreased calcification and prepared sections of long bones revealed narrowed epiphyses. The testes of the deficient animals were smaller by volume and showed fewer mitoses. The foregoing alterations, in the opinion of Harris and his co-workers, followed an inability of the animals to form adequate proteins. They believe that the changes are comparable to those seen in starvation.

In view of the paucity of information available to biochemists and morphologists it seemed desirable to gather data regarding the anatomic changes that occur in animals completely deficient in only one essential amino acid. In each experiment we have selected a group of young rats of a single strain of the same age and of comparable weight. The diet of the deficient rats was similar to that of a control group except that in each experiment it was deficient in a single amino acid. In the first experiment, recorded here the animals were deprived of phenylalanine.

## EXPERIMENTAL PROCEDURE

*Animals*—Weanling rats of the Sprague-Dawley strain, 25 to 28 days old, were employed in our experiment. Ten control animals were fed a complete synthetic diet containing a mixture of essential amino acids as the source of nitrogen, and 10 experimental animals were fed a diet similar in all respects except that phenylalanine was omitted. The paired feeding technique was employed. Each animal was kept in an individual cage designed to prevent coprophagy.

*Diets*—Both the control and the deficient diet consisted of salt mixture (Wesson<sup>6</sup>) 4 per cent, cellulose 2 per cent, sucrose 67.25 per cent, halibut oil 0.4 per cent, corn oil 3 per cent, and amino acids 23.35 per cent. The amino acid mixture<sup>7</sup> contained l-lysine<sup>8</sup>.

6 Wesson, L. G. *Science* **75** 339, 1932.

7 Frederick Stearns & Company supplied all the amino acids and vitamins required in these experiments.

8 This amino acid was administered in the form of a monohydrochloride.

\*Frederick Stearns & Company Fellow in physiologic chemistry.

From the Departments of Pathology and Physiological Chemistry, Wayne University College of Medicine.

1 Albanese, A. A., Holt, L. E., Jr., Kajdi, C. N., and Frankston, J. E. *J. Biol. Chem.* **148** 299, 1943.

2 Allen, E. *Sex and Internal Secretions*, ed. 2, Baltimore, Williams & Wilkins Company, 1939, chap. 22.

3 Albanese, A. A., Randall, R. M., and Holt, L. E., Jr. *Science* **97** 312, 1943.

4 Griffith, W. H., and Wade, N. J. *J. Biol. Chem.* **132** 627, 1940.

5 Harris, H. A., Neuberger, A., and Sanger, F. *Biochem. J.* **37** 508, 1943.



16 Gm, dl-tryptophan 32 Gm, l-leucine 128 Gm, dl-isoleucine 16 Gm, dl-valine 224 Gm, dl-methionine 96 Gm, l-arginine<sup>s</sup> 32 Gm, l-histidine<sup>s</sup> 64 Gm and dl-threonine 16 Gm per 500 Gm of diet. The control diet contained in addition 112 Gm of phenylalanine. In the phenylalanine-deficient experimental diet the aforementioned amount of phenylalanine was replaced isocalorically by sucrose. The diets were supplemented with 4 mg of thiamine hydrochloride, 8 mg of riboflavin, 4 mg of pyridoxine hydrochloride, 4 mg of nicotinic acid, 20 mg of calcium pantothenate, 600 mg of paraaminobenzoic acid, 50 mg of alpha tocopherol 2 Gm of inositol and 2 Gm of choline hydrochloride per kilogram. The average daily food intake was 2.53 Gm.

*Duration of Experiment*—The rats were maintained on the respective diets for twenty-eight days. Two deficient rats died several hours before they were to be killed (experiments 4 and 5).

At the termination of the experimental period the animals were killed by decapitation without anesthesia, since this procedure provided blood in sufficient quantities. From 10 to 15 cc was collected in an evaporating dish containing sodium oxalate. The amount of spinal fluid was negligible, as shown by an analysis made and compared with an analysis of blood from the abdominal portion of the aorta. After removal of samples for hemoglobin determinations, the remainder of the blood was centrifuged and determinations of plasma proteins carried out on the plasma.

*Autopsies*—Autopsies were made immediately following the death of the animals. Each organ was inspected and routinely weighed. The entire organs or sections of them were fixed in 4 per cent solution of formaldehyde. One each of the paired organs, as well as sections of the liver and the kidney, were fixed in Zenker's fluid. Sections of the sternum, the vertebrae and the long bones were decalcified in Zenker's fluid. The tissues were embedded in paraffin, sectioned and stained routinely with hematoxylin and eosin. Additional stains were employed as necessity demanded. Each eye was fixed in Zenker's fluid, one of them was embedded in paraffin, and the other in celloidin.

## RESULTS

*Health of the Deficient Animals*—The animals on the phenylalanine-deficient diets appeared to become progressively weaker throughout the experimental period. These rats were inactive and reacted slowly to stimuli. Their hair appeared coarse and unkempt. At the completion of the experimental period they were obviously smaller than the control rats, and their extreme weakness made it difficult for them to walk. Two animals (rats 4 and 5) died several hours prior to the time at which they were to be killed.

*Changes in Weight*—Although the amount of food consumed by the deficient and the control animals was the same (average, 2.53 Gm per day), the rats on the deficient diet did not maintain their weight, all showed a significantly greater loss in weight (average, 16 Gm) than did the control animals (average, 4.75 Gm).

*Organ Weights*—Each organ was weighed immediately after its removal, and the percentage of organ weight was determined. The percentage

of organ weight equals  $\frac{\text{organ weight}}{\text{body weight}}$ . The weights were compared in each experiment, and the totals were submitted to statistical analysis. The most pronounced variation was noted in the thymuses. The glands of the deficient animals

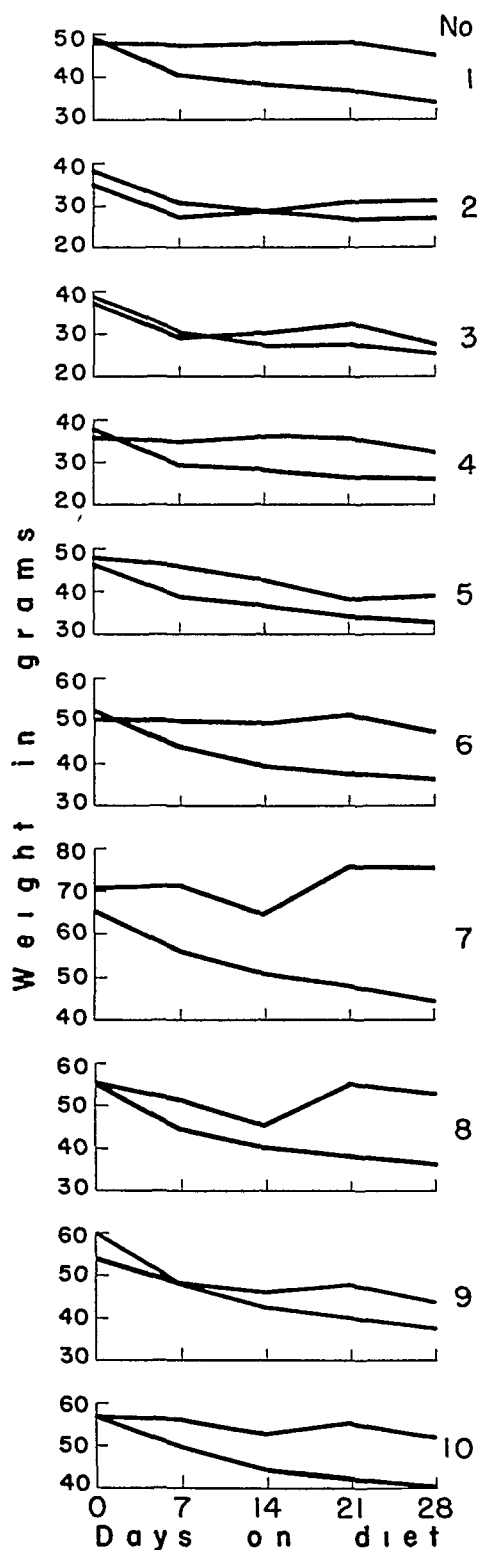


Fig 1—Comparison of the weight curves of each pair of rats, one deficient in phenylalanine, the other a control. The deficient animals weighed the least at end of experimental period.

proved to be markedly atrophic, the mean being  $21.2 \pm 2.9$  mg, compared with  $54.5 \pm 7.6$  mg for the control animals. The average organ weight of the deficient animals proved to be 0.115 per

cent, compared with 0.183 per cent for the control animals. The testes of the deficient rats proved to be uniformly smaller than those of the control group, the mean weight being  $95 \pm 7.6$  mg for the deficient animals and  $278 \pm 70$  mg for the control animals. The weights of the other organs showed no significant variations.

*Roentgen Studies*—In control rats the stomach and the lower gastrointestinal tract contained gas, while in the deficient rats these organs were relatively free of air. Other than a difference in the length of long bones, there were no skeletal differences in the control and deficient groups.

*Hemoglobin*—Determinations of hemoglobin were made by the Sheard-Sanford method.<sup>9</sup> The hemoglobin was consistently lower in animals on the phenylalanine-free diet, ranging from 7.4 to 14.0 Gm per hundred cubic centimeters of blood, with an average of 9.9 Gm, animals on the complete diet had hemoglobin levels ranging from 13.7 to 15.8 Gm, with an average of 14.7 Gm.

*Plasma Protein*—Plasma protein was determined by the micro-Kjeldahl method with the aid of a Pregl<sup>10</sup> distillation apparatus. In the deficient animals there was a reduction of the plasma protein with an average of 4.71 Gm per hundred cubic centimeters, compared with 5.58 Gm in the control animals. From these data it would appear that phenylalanine influences both hemoglobin and plasma protein formation.

*Hepatic Fats*—The fats of the liver were determined essentially according to the method of Leathes and Raper.<sup>11</sup> The hepatic fat of the control animals proved to be 4.56 Gm per hundred grams which was not significantly different than that of the deficient animals (3.97 Gm).

#### MORPHOLOGIC OBSERVATIONS

*Heart and Blood Vessels*—The entire heart was bisected, and sections of the complete organ were prepared. Sections obtained from the aorta and from the blood vessels of the lungs, the kidneys and the peripheral muscles of each animal were examined. No alterations were observed in the cardiac muscle, and the blood vessel walls of the deficient animals appeared to be comparable with those of the controls.

*Lungs*—Complete sections of both lungs were examined. The lungs of both the control and the deficient animals proved to be normal save for the lungs of 2 deficient rats that died on the day they were to be killed. Nodular masses in these lungs were noted on the gross specimens, masses that on histologic examination proved to be focal areas of bronchopneumonia.

*Liver*—The livers of the two groups of animals were comparable and presented no abnormalities.

*Spleen*—The spleens of the two groups presented no gross abnormalities, but those of the deficient animals showed congestion of the pulp. The structure and the cells appeared normal.

*Kidneys*—On gross inspection the kidneys of both deficient and control animals appeared normal and comparable in size. However, the kidneys of the deficient animals were somewhat increased in weight. Inspection of the glomeruli and tubules and the vessels revealed no alterations in either the deficient or the control group.

*Urinary Bladder*—The urinary bladders of both the control and the deficient animals were normal.

*Gastrointestinal Tract*—On gross inspection the gastrointestinal tracts of both groups of animals appeared to be normal. Sections were obtained from both portions of the stomach, from three levels of the small bowel and from the several levels of the large bowel. The mucosa was well preserved in all animals and showed no histologic alterations.

*Skin*—The hair of the deficient animals was stringy and coarse to palpation. Inspection of the skin showed an increased amount of subcutaneous fat and connective tissue in the control group. Examination of the prepared sections taken from the ventral and lateral abdominal walls showed the epidermis and the corium to be comparable in the two groups. Although no detailed studies were made, there were no obvious differences in the appearance of the hair follicles.

*Voluntary Muscle*—The muscles of the deficient group of animals appeared similar to those of the control animals, although they were somewhat smaller. Histologic study of the muscles revealed no alterations in either group.

*Bones and Joints, Marrow*—Examination of longitudinal sections of the tibia and the femur, softened in Zenker's fluid, revealed these facts: 1. The epiphyseal cartilages of the deficient rats were consistently narrower than those of the control animals. To check this observation the

<sup>9</sup> Sheard, C., and Sanford, A. H. J. Lab. & Clin. Med. **14**: 558, 1929.

<sup>10</sup> Pregl, F. Die quantitative organische Mikro-analyse, ed. 2, Berlin, Julius Springer, 1912.

<sup>11</sup> Leathes, J. B., and Raper, H. S. The Fats, ed. 2, New York, Longmans, Green & Co., 1925.

width of the cartilage disk was measured at three zones. The average width of the disk for the 10 deficient rats proved to be 0.116 mm, compared with an average of 0.174 mm for the control animals. The cartilage cells in the deficient rats showed no alterations, but the columns of cells in the control group were more uniformly

in the deficient animals. 2 The bony trabeculae appeared more prominent and larger in the control animals. This difference was not striking, and the degree of calcification appeared equal in both groups.

Marrow was available for examination from the long bones, the sternum and the vertebrae

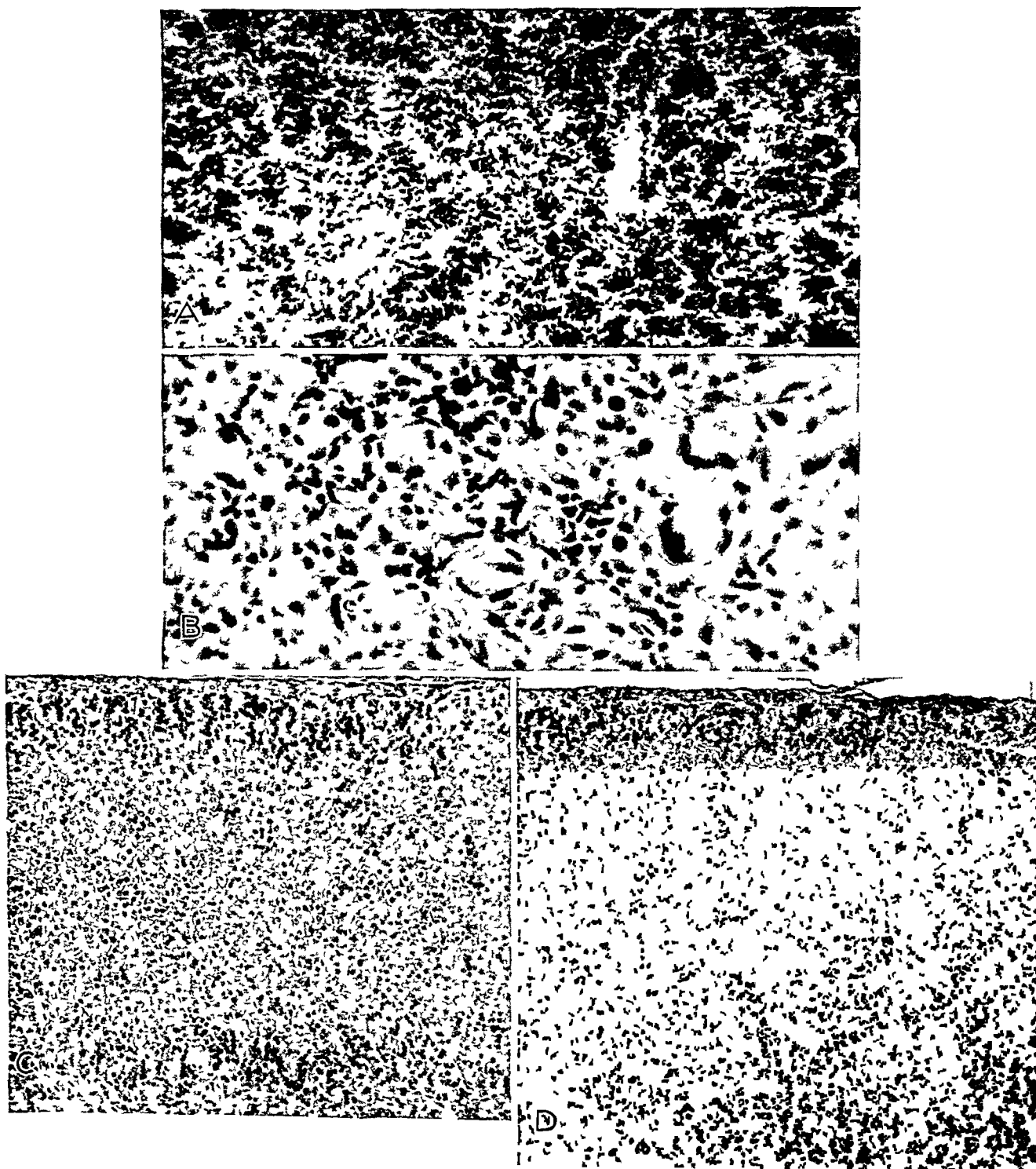


Fig 2—A, thymus from the control animal of pair 1. B, thymus from the deficient animal of pair 1. Hematoxylin and eosin,  $\times 80$ . C, adrenal gland from the control animal of pair 7. Hematoxylin and eosin,  $\times 150$ . D, adrenal gland from the deficient animal of pair 7. Hematoxylin and eosin,  $\times 150$ .

arranged. This alteration along with the presence of islands of cartilage encircled by bony trabeculae on the joint margins of the cartilage produced some irregularity of the epiphysial line

There were no alterations in the marrow specimens of either group.

*Brain, Spinal Cord and Nerves*—In each instance the fixed brain was sectioned so that the

entire organ was available for study. Sections of the spinal cord were taken from at least three levels, and peripheral nerves were studied in muscles taken from the legs. No alterations were observed in the central or the peripheral

*Pituitary Gland*—In an attempt to preserve the pituitary gland intact the base of the skull and the attached pituitary gland were fixed in Zenker's fluid. Unfortunately, it was difficult to prepare sections of the gland with this prepa-



Fig 3—*A*, testis from the control animal of pair 2, *B*, testis from the deficient animal of pair 2. Note sloughing of the epithelium in the lumens. Hematoxylin and eosin,  $\times 150$ . *C*, section of a femur of the control animal of pair 5, *D*, section of a femur of the deficient animal of pair 5. Hematoxylin and eosin,  $\times 80$ .

nervous system of any of the normal or the deficient rats.

*Eyes*—Neither the eyes of the deficient nor those of the control animals showed alterations

ration, so only four glands from the deficient and control groups were available for examination. The sections were cut at 4 microns and stained with hematoxylin and eosin and with

azocaine. No alterations were noted in either the deficient or the control group.

*Thyroid and Parathyroid Glands*—Because the animals were decapitated it was difficult to find the thyroid gland. However, by preparing numerous sections from the soft tissues of the neck the gland was found in 2 rats of the deficient group and in 3 rats of the control group. In all a parathyroid gland was embedded in the thyroid tissue. No differences were noted between the glands of the two groups.

*Thymus and Lymph Nodes*—At autopsy the thymus of each of the deficient rats was found to be extremely small and in some instances almost impossible to identify, while the thymus of each of the control animals filled the superior mediastinum.

Examination of the prepared sections showed the structure of the thymuses from the control animals to be well preserved and the cellular elements normal. The obvious atrophy of the glands of the deficient animals was more striking on histologic study. The atrophy varied from moderate to severe, so that in some instances only remnants of the gland could be identified. The earliest changes were represented by thinning of the lymphoid elements of the cortical zone with resultant narrowing. As these lymphoid elements became more sparse the connective tissue stroma and the reticulum became relatively more prominent. In those glands presenting severe atrophy, only a few lymphocytes were scattered through a thin cortex, while the medullary regions formed the bulk of the organ. In addition to the apparent relative increase in stroma, proliferation of the reticular cells was observed, and numerous giant cells were noted in mature and apparently newly formed stroma. The central portions of such lesions contained numerous irregular clefts typical of cholesterol deposits. The giant cells were often of the foreign body but more frequently of the Langhans type. The formed granulomas were most prominent in the glands that were markedly atrophic, only a few giant cells could be found in the more normal glands.

Lymph nodes were obtained from the axilla, the mediastinum and the neck. The structure of all was well preserved and no abnormalities were found in the nodes removed from either the control or the deficient groups of animals.

*Adrenal Glands*—Although the adrenal glands from both groups of animals were on gross examination almost equal in weight, histologic study revealed noticeable changes in the glands of the deficient rats. These alterations varied

with each animal and were observed only in the cortex. It was apparent by examination under low magnification that the structure was usually well preserved but that thinning of the cortex was often present. The intermediate zone almost uniformly appeared to be crowded or compressed as compared with that of the control animal. In addition, numerous nuclei of the cells of the intermediate zone appeared as deeply stained dense granular masses. More careful inspection of these nuclei disclosed a few thin spirals and often chromatin clumps. These may represent atypical or abortive mitoses. Other hypertrophied nuclei showed prominent nucleoli and large masses of chromatin. Further inspection of the intermediate zone under high magnification showed the cells to be smaller and to contain less lipid. The intervening vascular channels were more prominent here and also in the reticular zone. In deficient animals the cellular compression and the prominent vascular spaces were probably produced by cellular atrophy.

*Pancreas*—The pancreases of the two groups of animals were comparable and normal.

*Female Genital System*—The ovaries, oviducts and uteri were available from 3 animals of the deficient group and 3 animals of the control group. Admittedly, evaluation of these tissues is difficult, but no differences were observed in either group. It was noted that maturation of follicles was present in animals of the deficient group.

*Male Genital System*—Testicular atrophy was prominent in most animals maintained on the phenylalanine-deficient diets. Examination of the prepared slides presented more striking changes. The tubules of the testes of the deficient animals appeared smaller, and the cellular alterations were prominent. Large eosinophilic-staining cells and considerable cellular detritus were found in the lumens of the atrophic tubules. Few cells had developed beyond the stage of the primary spermatocytes and the Sertoli cells were prominent. The interstitial cells and the cells of the epididymis showed no prominent alterations. Unfortunately, the prostate gland and seminal vesicles were not removed for examination.

#### COMMENT

Some of the anatomic alterations observed in phenylalanine-deficient rats have previously been observed in experiments on chronic and general malnutrition<sup>12</sup>. It has been observed that under-

<sup>12</sup> Jackson, C. M. *The Effects of Inanition and Malnutrition upon Growth and Structure*, Philadelphia, P. Blakiston's Son & Co., 1925.

fed rats show a retardation of skeletal growth and that rats fed on lysine-deficient<sup>5</sup> diets have narrowed epiphyses and a decrease of calcium in their bones, the latter not observed in these studies, however. Profound thymic atrophy has been frequently noted in both man and animals following acute and chronic inanition, and the decrease observed in the lymphoid portions of the gland apparently simulates the atrophy noted here in phenylalanine-deficient rats. Atrophy of the adrenal cortex<sup>13</sup> with a decrease in the lipid content of the cortical cells has also been noted, differing little from that observed in the present experiment. Retrogressive alteration in the epithelial cells of the seminiferous tubules in the phenylalanine-deficient rats and in those reported on starvation diets is less severe than the alteration in vitamin E<sup>14</sup> deficiency but about equal to that in vitamin A deficiency.

13 Jackson, C. M. *Am J Anat* **25** 221, 1919

14 Mason, K. E. *Am J Anat* **52** 153, 1933

#### SUMMARY

Young rats were fed synthetic diets of crystalline amino acids, crystalline vitamins, fats and dextrin plus the necessary salts. The animals were pair fed so that the rat of the control group received the exact quantity of food that his previously fed paired partner of the deficient group received. The control animals received a balanced diet while those of the deficient group received a diet devoid of phenylalanine but otherwise identical with the control diet. During the experimental period of twenty-eight days the animals of the deficient group lost weight, gradually became weaker and in general appeared unkempt.

Those rats fed on diets deficient in phenylalanine showed these results: a reduction in hemoglobin and plasma proteins, narrowing of the epiphysal cartilages of the long bones, marked atrophy of the thymus, atrophy and decreased lipid content of the adrenal cortex, degeneration and atrophy of the seminiferous tubules of the testes.

# PATHOLOGY OF ANAPHYLAXIS DUE TO SULFONAMIDE DRUGS

B BLACK-SCHAFFER, MD

RICHMOND, VA

Systematic study of the pathology of human anaphylaxis is in its infancy. Most of the recognized instances of anaphylactic death are of the sudden, dramatic variety, with no time for the appearance of morphologic changes. Some common, as well as some rare, diseases are believed to be of allergic origin, but the proof is either incomplete or disputed, rheumatic fever, periarteritis nodosa, rheumatoid arthritis, acute pancreatitis, lupus erythematosus disseminata, glomerulonephritis, scarlet fever and infectious mononucleosis are but a few examples. All have been inculpated because of clinical or historical similarities to serum sickness or accelerated allergic responses in both man and animals and because of the anatomic changes noted from time to time in material obtained at autopsies. These data to date have been totally inadequate for the construction of a well documented catalog of human immunopathology. This lack has blocked and continues to block a wider appreciation and acceptance of the anaphylactic interpretation of disease.

The universal use of sulfonamide compounds and the discovery that they can act as antigens capable of eliciting fatal reactions have, for the first time, made possible the study of a large number of relatively slow but fatal anaphylactic reactions. Because of these circumstances, the lesions discovered and the problems suggested by 5 cases of anaphylactic death following therapeutic use of sulfonamide compounds are presented.

## REVIEW OF LITERATURE

The phenomenon of anaphylactic death has fascinated immunologists and some pathologists since Portier and Richet<sup>1</sup> recognized the significance of the fulminating fatal syndrome induced in some of their dogs. The anatomic changes accompanying this type of profound shock and subsequent death in both animals and man are so limited by the rapidity of the

reaction that only the demonstration of a passive transfer of sensitivity (Prausnitz-Kustner reaction) in conjunction with an evaluation of the past history and the circumstances of death can establish the cause of the sudden death.

The production of local lesions by intrinsically harmless proteins (Arthus<sup>2</sup>) opened the door to the anatomic study of both local and generalized anaphylaxis. Since then the results of many investigations of the morphologic evidence of antigen-antibody reaction have been published, and numerous variations of Richet's and Arthus' fundamental experiments have led to the recognition of certain lesions as characteristic of (if not specific for) the hyperergic tissue response.

Von Pirquet<sup>3</sup> and Shick described in accurate detail a "unique" anaphylactic reaction in man which occurred eight to twelve days after a primary injection of horse serum. The syndrome was marked by a high temperature, cutaneous rashes (sometimes morbilliform or scarlatiniform), a tendency toward leukopenia, transient arthritis, lymphadenopathy, subcutaneous edema and albuminuria. This clinical process they named *Serumkrankheit* (serum sickness).

When, in 1917, Landsteiner<sup>4</sup> and Lampl demonstrated the mechanism whereby nonallergenic substances (simple chemical compounds) became allergens, the basis of a real understanding of drug allergy was laid.

Allergic reactions elicited by nonprotein drugs have been described so often and so regularly in medical journals that few well informed persons were skeptical when, in 1937, Hageman and Blake<sup>5</sup> reported the serum-sickness-like reactions of a group of patients who were treated with sulfanilamide. Goodman and Levy<sup>6</sup> were among the first to suggest that the rash appear-

From the Department of Pathology, Medical College of Virginia.

<sup>1</sup> Portier and Richet, C. *Compt rend Soc de biol* **54** 170, 1902.

<sup>2</sup> Arthus, M. M. *Compt rend Soc de biol* **55** 817, 1903.

<sup>3</sup> von Pirquet, C. *Jahrb f Kinderh* **62** 537, 1905.

<sup>4</sup> Landsteiner, K. *The Specificity of Serological Reactions*, Springfield, Ill., Charles C Thomas, Publisher, 1936.

<sup>5</sup> Hageman, P. O., and Blake, F. G. *J A M A* **109** 642, 1937.

<sup>6</sup> Goodman, M. H., and Levy, C. S. *J A M A* **109** 1009, 1937.



ing after treatment with sulfanilamide was the result of hypersensitivity, and while cutaneous tests in their 2 cases were respectively negative and doubtful, readministration of a small dose of the drug by mouth promptly resulted in a typical itching reaction of the skin. Schlesinger and Mitchell<sup>7</sup> studied the course of eruptions in children treated with sulfanilamide and noted a "natural history" of the rash marked by prodromal fever, transient leukopenia and a morbilliform and occasionally a scarlatiniform rash often associated with splenomegaly, increased capillary fragility and in some cases arthritic symptoms.

Schonholzer<sup>8</sup> demonstrated that azosulfamide is bound to the serum albumin, and Davis<sup>9</sup> succeeded in conjugating sulfonamide compounds to human blood serum by procedures following the methods pioneered by Landsteiner and Lampl. The results were confirmed by Wedum<sup>10</sup> and Gerber and Gross<sup>11</sup>. Wedum, using human and rabbit serum, as well as egg albumin, as the protein portion of the antigenic molecule was able to produce anaphylactic shock, positive intradermal reactions and, furthermore with appropriately prepared antibodies, *in vitro* precipitation of the antigen. Gerber and Gross likewise demonstrated the antigenicity of conjugated sulfonamide compounds by producing anaphylaxis and the Shwartzman phenomenon in guinea pigs and rabbits.

Schlesinger and Mitchell,<sup>7</sup> using conjugated sulfanilamide, encountered the difficulties that had been met by past investigators in the field of drug allergy. They were unable to elicit in hypersensitive patients a positive reaction with scratch, patch or intradermal tests. Attempts to demonstrate hypersensitivity by means of the Prausnitz-Kustner reaction likewise failed. Because of these difficulties, doubt was expressed by many as to the validity of the anaphylactic concept of the reactions following treatment with sulfonamide compounds. More recently, however, Shaffer, Lentz and McGuire<sup>12</sup> have reported the production of a positive Prausnitz-Kustner reaction to sulfathiazole with serum from persons who had reacted to the drug a second time.

Leftwich<sup>13</sup> has done much to dispel any remaining skepticism by utilizing as test material the blood serum of patients receiving a sulfonamide drug in the course of therapy. Reasoning that the drug must be present in the blood stream as a protein conjugate, he inoculated intradermally 0.05 cc of a serum known to contain conjugates into 30 persons who had reacted to sulfonamide compounds with a typical hypersensitive pattern. Twenty-eight of these persons promptly responded to the homologous sulfonamide conjugate by wheal formation at the site of inoculation, 2 did not react. As a control 26 persons who had received treatment without reacting were tested in a similar manner. 24 showed no reaction, and only 2 reacted. The specificity of the cutaneous reactions was demonstrated by the fact that only 3 of the sensitive patients reacted to a nonhomologous conjugate. This form of cross sensitization was also encountered by Wedum<sup>10</sup> and by Gerber and Gross<sup>11</sup> in their experimental work and has been observed in the course of human therapy.

Since morphologic changes which are considered characteristic of anaphylactic states have been noted in immunopathologic experiments it was natural that similar lesions should be sought in persons who died during or after reactions of an allergic type following treatment with sulfonamide compounds. Numerous isolated instances and a few series of cases of death due to the effects of sulfonamide drugs have been reported in the literature, most of the authors have been concerned with the mechanism of urolithiasis medicamentosa or with the fatal blood dyscrasias agranulocytosis and hemolytic anemia. Since mechanical uremia lies outside the scope of this paper, it will not be further considered. The two blood dyscrasias are yet to be encountered in the autopsy room of the Medical College of Virginia, and only their minimal and theoretic expressions leukopenia and erythrophagocytosis, will be discussed.

Cutts, Burgess and Chafee<sup>14</sup> encountered one death in a series of patients treated with sulfathiazole. On the eighth day of therapy a generalized rash appeared. Because of developing oliguria, the drug was discontinued on the eleventh day. However, the secretion of urine decreased to total anuria. In the autopsy protocol the kidneys were said to have shown tubular degeneration with foci of necrosis and interstitial round cell infiltration containing some neutrophilic poly-

7 Schlesinger, E. R., and Mitchell, W. L. *Am J Dis Child* **56** 1256, 1938.

8 Schonholzer, G. *Klin Wchnschr* **19** 790, 1940.

9 Davis, D. B. *Science* **95** 78, 1942.

10 Wedum, A. J. *J Infect dis* **70** 173, 1942.

11 Gerber, I. E., and Gross, M. *J Immunol* **48** 103, 1944.

12 Shaffer, B., Lentz, J. W., and McGuire, J. A. *J A M A* **123** 17, 1943.

13 Leftwich, W. B. *Bull Johns Hopkins Hosp* **74** 26, 1944.

14 Cutts, M., Burgess, A. M., and Chafee, F. H. *New England J Med* **223** 762, 1940.

mononuclear leukocytes. This was one of the earliest cases in which anuria was reported as not due to mechanical obstruction.

Erganian and Doval<sup>15</sup> had a similar experience with a child in whom anuria developed following readministration of sulfathiazole. The kidneys showed marked tubular degeneration, and although concretions were present in the pelvises, the authors believed that the anuria was due in large part to the tubular damage.

Monto<sup>16</sup> described 3 cases of chemotherapeutic anuria encountered within a relatively short time at the Henry Ford Hospital. He was convinced that the failure of the kidneys was due to the nephrotic changes and not to crystalluria.

Since the lesions of the fatal reactions are generalized and not limited to the kidneys, the paper by Lederer and Rosenblatt,<sup>17</sup> soon followed by that of Merkel and Crawford,<sup>18</sup> gave the first detailed and balanced morphologic picture of such reactions. These authors were not primarily concerned with the problem of anaphylaxis, confining themselves to anatomic descriptions, although Lederer and Rosenblatt suggested that anaphylaxis might in part account for the nonbacterial foci of necrosis which stood in the foreground of the observed changes. These foci were surrounded by cellular exudates whose components, monocytes, lymphocytes and neutrophilic leukocytes, varied in proportion from case to case, with the monocytic elements predominating. They were described in the following organs: liver, spleen, lymph nodes, marrow, lungs, heart, kidneys, adrenal glands and pancreas. In addition, the convoluted tubules of the kidneys were so degenerated as to justify application of the term "nephrosis" in most cases. Interstitial myocarditis was observed by Lederer and Rosenblatt in 2 instances, accompanied in case 3 by a heavy monocytic mantling of some of the coronary arteries.

No characteristic pulmonary lesion was encountered, foci of consolidation and necrosis were seen.

The liver was the most frequent site of foci of necrosis, these were small and left the structure of the organ essentially intact. Kupfer cells were prominent, and in case 2 the portal fields were markedly infiltrated by monocytes and occasional neutrophilic leukocytes.

In every instance in which the spleen was histologically examined it contained areas of ischemic necrosis. Identical lesions of the lymph nodes were particularly well described by Merkel and Crawford.<sup>18</sup>

Rich<sup>19</sup> recognized the relationship between lesions like those of periarteritis nodosa which he observed in 6 patients who had received massive serum therapy and the serum sickness which preceded the death of 5. A seventh patient, receiving no serum but only sulfonamide drugs, showed the same lesions as the preceding 6. This observation was confirmed a short time later by investigation of a second instance of reaction to a sulfonamide compound.<sup>20</sup> The lesions seen by Rich were primarily vascular and consisted of fibrinoid necrosis of the walls of small arteries, which had rich adventitial collars of monocytes and occasional polymorphonuclear leukocytes. In addition in 3 of the cases of serum sickness interstitial myocarditis and focal necrosis of the lymph nodes were noted. In the 2 cases of reaction to sulfonamide compounds aseptically, widely distributed foci of necrosis of the type described by Lederer and Rosenblatt<sup>17</sup> were seen, furthermore in the second of these 2 cases the same type of interstitial myocarditis, interstitial nephritis and a unique form of pancreatitis were observed. The infiltrating cells in all organs were chiefly monocytes, lymphocytes and neutrophilic and occasionally eosinophilic polymorphonuclear leukocytes.

Many of these lesions had previously been described by numerous authors studying experimental anaphylaxis in animals.

The latest contribution in this field is the work of Rich and Gregory<sup>21</sup> who induced what Fleischer and Jones<sup>22</sup> first described as the equivalent of serum sickness in rabbits. Post-mortem study of these animals revealed all the characteristic changes of protein anaphylaxis,<sup>23</sup> the lesions being comparable in appearance and distribution to those observed by Rich in human serum sickness and reaction to sulfonamide drugs.

19 Rich, A. R. Bull. Johns Hopkins Hosp. **71** 123, 1942.

20 Rich, A. R. Bull. Johns Hopkins Hosp. **71** 375, 1942.

21 Rich, A. R., and Gregory, J. E. Bull. Johns Hopkins Hosp. **72** 65, 1943.

22 Fleischer, M. S., and Jones, L. J. Exper. Med. **54** 597, 1931.

23 (a) Hemlein, H. Ergebn. d. Hvg., Bakt., Immunitätsforsch. u. exper. Therap. **20** 274, 1937. (b) Klinge, F. Beitr. z. path. Anat. u. z. allg. Path. **83** 185, 1929. (c) Vaubel, E. ibid. **89** 374, 1932. (d) Apitz, K. Virchows Arch. f. path. Anat. **289** 46, 1933.

15 Erganian, J. A., and Doval, J. H. J. Lab. & Clin. Med. **28** 808, 1943.

16 Monto, R. W. Ohio State M. J. **38** 925, 1942.

17 Lederer, M., and Rosenblatt, P. J. A. M. A. **119** 8, 1942.

18 Merkel, C., and Crawford, R. C. J. A. M. A. **119** 770, 1942.

Gessler<sup>24</sup> has recently contributed a description of 3 cases of death caused by reaction to sulfonamide compounds, in which the pathologic changes were similar to those just described.

No attempt has been made to review extensively the immense literature of anaphylaxis and reactions to sulfonamide compounds. However, a number of excellent reviews are available and are listed among the references.<sup>25</sup>

#### REPORT OF CASES

**CASE 1**—A 23 year old Negro woman was given sulfathiazole by her physician because of abdominal pain. She rapidly grew worse, and after four days was referred to St Philip's Hospital. In the emergency room her appearance of extreme shock was confirmed by a blood pressure reading of 70 systolic and 50 diastolic. Her temperature was recorded as 105 F, and the pulse rate as 110 per minute. A blood count revealed 4,800,000 erythrocytes and 12,650 leukocytes per cubic millimeter. The urine showed albumin (3 plus) and rare hyaline casts. Chemical studies of the blood were reported as showing nonprotein nitrogen 190 mg, sugar 143 mg and sulfathiazole 18 mg per hundred cubic centimeters. The patient died the day after admission to the hospital. The clinical diagnosis was uremia due to sulfathiazole.

**Gross Examination**—The spleen weighed 190 Gm. Its capsule was covered by a thin layer of fibrin and was marked by small, prominent, firm, confluent brown-red areas, which on section showed pyramidal configuration with the bases on the capsule. The surrounding tissue was not remarkable in appearance.

The left fallopian tube was bound to its ovary by fibrous adhesions, the fimbriae were accreted and sealed the lumen.

The right and left kidneys weighed respectively 220 and 200 Gm, the fibrous capsules stripped with ease, revealing smooth lobulated surfaces. Sections showed pale bulging surfaces with well demarcated corticomedullary boundaries. The pelves were not remarkable.

**Microscopic Examination**—The myocytes of the heart were fragmented, and the intervening spaces were widened by edema. A sparse interstitial monocytic infiltration was present, particularly prominent about the small vessels.

Sections of liver were characterized by early fragmentation of the cords. The portal fields were infiltrated by monocytes, eosinophilic leukocytes and occasional plasma cells. Stille's spaces were wide, the sinusoids were moderately congested and contained occasional macrophages, which were filled with leukocytes (fig 1) and erythrocytes. The parenchymal cytoplasm was the seat of cloudy swelling. Kupffer's cells were prominent.

The follicles of the spleen were enlarged and showed marked central hyperplasia and necrosis. The reticuloendothelium was hyperplastic and displayed active erythrophagocytosis and leukophagocytosis. The firm pyramidal zones already described grossly were areas of ischemic necrosis. Necrobiotic neutrophilic leukocytes and much basophilic debris marked the areas of necrosis, which were demarcated by a zone of reaction.

Lymph nodes from various sites showed diffuse hyperplasia, some contained macrophages similar in all respects to those described in the spleen. Numerous eosinophilic and neutrophilic polymorphonuclear leukocytes were scattered about the pulp.

The renal interstices were infiltrated by monocytes, plasma cells and occasional eosinophilic leukocytes. These showed a tendency toward focalization about glomeruli and small vessels. The glomerular tufts contained little blood, and in a few instances the number of endothelial cells appeared to be increased. The glomerular spaces contained precipitated protein, and the parietal lining was composed of cuboidal cells. The tubules showed cloudy swelling of their epithelium but no necrosis. Examination of the peripelvic fat clearly showed the ubiquitous exudate described in the heart, the liver and the kidneys.

**Pathologic Diagnosis**—Interstitial myocarditis, serous hepatitis, interstitial nephritis, focal splenic necrosis (Fetis), generalized lymphadenitis, salpingitis on the left side, erosions of the cervix, cavernous hemangioma of liver.

**CASE 2**—A white man 54 years old was referred from a tuberculosis sanatorium to the Medical College of Virginia Hospital for treatment of "vague urinary disturbances" and abdominal discomfort of three months' duration. On admission the temperature, the pulse and respiratory rates and the blood pressure were well within normal limits. The urine revealed no albumin and no sugar, the blood count was not remarkable, and the blood nonprotein nitrogen was reported as 36 mg per hundred cubic centimeters. A phenolsulfonphthalein test for renal function resulted in the excretion of 53 per cent of the injected total in two hours and twenty-five minutes. The unusual interval of time for this procedure was entailed by the patient's difficulty in micturition, due to an enlarged prostate gland.

Physical examination did not produce any clinical evidence of disease other than prostatic hypertrophy.

For some unrecorded reason, 240 grains (15.5 Gm) of sulfathiazole was prescribed and administered from the day of his admission—May 19 to May 23, 1943.

Roentgenologic investigation of the kidneys on May 22 revealed an enlarged minor upper calyx in the left organ. Urine from the corresponding ureter contained 3 to 4 erythrocytes per high power field. The contralateral kidney excreted normal urine. After the first few days of sulfathiazole medication the patient frequently vomited, refused food and was given large amounts of fluid intravenously. On May 29 he complained that he had not voided for five or six days. Blood taken for chemical studies on May 28 contained 112 mg of nonprotein nitrogen and 80 mg of creatinine per hundred cubic centimeters.

The temperature, which had been normal up to May 29, began to rise and remained at a level of about 102 F for the remainder of his stay.

On May 30 and 31 he was given 30 grains (2 Gm) of sulfathiazole intravenously in a desperate attempt to stave off death, which occurred thirteen days after his admission.

**Gross Examination**—The left lung weighed about 750 Gm. Fibrinous bands bound the parietal and the visceral pleura in the axillary line. The organ was subcrepitant throughout except for some marginal emphysema. Section brought to view foci of consolidation in both lobes. The right lung weighed 500 Gm and was essentially similar to the contralateral organ. The bronchial lymph nodes draining the right lung were enlarged and contained calcific concretions and soft caseous gray-white material.

<sup>24</sup> Gessler, C. N. South M. J. **37** 365, 1944.

<sup>25</sup> Ratner, B. Allergy, Anaphylaxis and Immunotherapy, Baltimore, Williams & Wilkins Company, 1943. Longcope, W. T. Medicine **22** 251, 1943. Simon, M. A. Am J M Sc **205** 439, 1943. Heinlein.<sup>23a</sup>

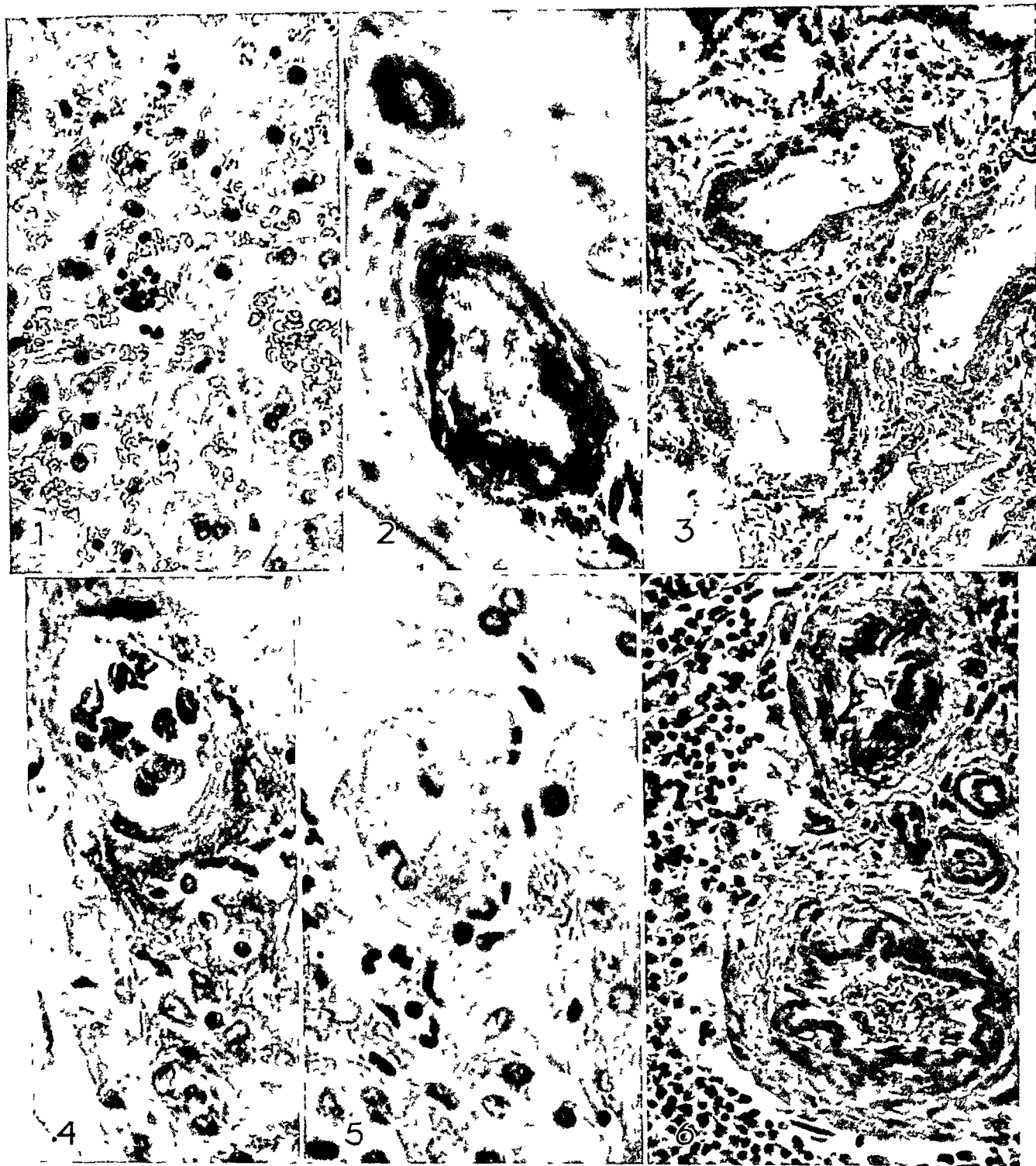


Fig 1 (case 1)—Serous hepatitis Trabeculae are moderately fragmented, Disse's spaces are prominent, and within a sinusoid is a macrophage filled with leukocytes ( $\times 441.6$ )

Fig 2 (case 2)—Fibrinoid necrosis of a small coronary artery ( $\times 441.6$ )

Fig 3 (case 2)—Interstitial pneumonia The infiltrating leukocytes lie within edematous septums, the alveoli are outlined by fibrin membranes ( $\times 165.6$ )

Fig 4 (case 2)—Necrotizing arteritis in renal pelvis and fibrinoid degeneration of the adjacent connective tissue ( $\times 653$ )

Fig 5 (case 2)—Necrosis of an isolated renal convoluted tubule surrounded by leukocytes The epithelium of neighboring tubules shows marked degeneration ( $\times 653$ )

Fig 6 (case 2)—Intimal fibrinoid degeneration of splenic arteries and arterioles ( $\times 331$ )

The prostate gland was moderately enlarged, and its median lobe impinged on the trigonum vesicae. The bladder contained no urine, and its mucosa was edematous. A small calculus obstructing the left ureteral orifice was removed, revealing uninjured mucosa.

The left ureter and the renal pelvis were somewhat dilated but, except for one calix, were everywhere lined by normal-appearing gray mucosa. The calix excepted measured about 2 cm in diameter and was lined by black-stained tissue. Its lumen was partly filled with similar-colored viscid material, no calculi were seen. The capsules of both kidneys stripped with moderate difficulty, revealing coarsely granulated surfaces. On section, the tissue planes bulged and were livid gray. Structural details were obscured, and the corticomedullary boundary was poorly defined.

*Microscopic Examination*—The myocardial fibers were separated by marked interstitial edema, they were fragmented but not necrotic. The small arteries were marked by homogeneous swelling of the intima and vacuolation of the media (fig 2). The nuclei of the myocytes in some vessels were distorted, some showing pyknosis and others karyorrhexis. A minimal exudate of monocytes was present in the interstices, chiefly about the vessels.

Study of numerous sites in all pulmonary lobes revealed edematous alveolar septums, infiltrated by mononuclear cells, many of which were frank macrophages, containing vacuoles and occasionally a leukocyte, an erythrocyte or both. Numerous interalveolar arterioles and capillaries were filled with conglutinated thrombi. The alveolar walls were outlined by acidophilic fibrin membranes, which characterized the lesion (fig 3). Within the air sacs was an exudate consisting largely of macrophages, monocytes, fibrin and neutrophilic leukocytes. The proportions of these elements varied from one region to another. Nowhere, however, were the polymorphonuclear leukocytes numerous. Large macrophages, in some loci assuming the proportions of giant cells, filled entire alveolar groups. Many of the pulmonary arteries showed medial vacuolation and subintimal leukocytic exudate.

The renal structure was intact. The abscess of the calix in the left kidney was lined by granulation tissue infiltrated by macrophages, its lumen containing numerous neutrophilic leukocytes and monocytes, the process was sharply delimited from the renal parenchyma. Sections of other calices revealed completely intact epithelium of the transitional type. The submucosa was diffusely infiltrated by monocytes, lymphocytes and occasional eosinophilic leukocytes. Small arteries, whose walls showed the changes described in the heart, were likewise present. Some of the vessels were frankly necrotic, and the surrounding connective tissue was edematous, intensely eosinophilic and infiltrated by monocytes (fig 4). This form of fibrinoid connective tissue necrosis was focal in distribution. Both organs contained numerous small fibrous cortical scars, which were subcapsular, and contained completely fibrotic glomeruli. The tubules within these foci were atrophic, and the interstices were infiltrated by lymphocytes. A second exudate of different composition—monocytes, plasma cells, eosinophilic leukocytes and occasional neutrophilic leukocytes—was diffusely spread throughout the interstices of both the cortex and the medulla. The glomeruli on the whole were relatively avascular. No increase in endothelial or epithelial cells of the capillary loops was apparent, however, the cells of the parietal leaf of Bowman's capsule were hypertrophied, some having assumed almost cuboidal appearance. Isolated convoluted tubules were necrotic (fig 5), the

greatest number showed only marked cloudy swelling. No evidence of tuberculosis was seen in either kidney. Arterial medial edema and nuclear changes of the type already described were again encountered.

A section of the aorta showed marked fibrous thickening of the intima, which contained foci of atheromatosis, adherent was a laminated thrombus. The subjacent intima was infiltrated by neutrophilic polymorphonuclear leukocytes, at the junction of the intima and the media was an exudate of monocytes and polymorphonuclear leukocytes invading the innermost zone of the media. Within this region the myocytes were edematous and the nuclei distorted. The adventitia was the seat of a similar but moderate cellular exudate.

The sinusoids of the spleen were markedly congested, the follicles were enlarged and contained numerous neutrophilic leukocytes. The reticuloendothelial cells were hyperplastic, and some germinal centers were necrotic. Fibrinoid homogenization of the intimal coat of the arterioles was seen in almost all follicles (fig 6). Infiltrating beneath the endothelium of some of the larger vessels were numerous monocytes, lymphocytes and rare polymorphonuclear leukocytes.

The centrilobular sinusoids of the liver were dilated and congested. The parenchymal cells bordering these areas were compressed and their cytoplasm was granular. Kupffer cells were hypertrophied and Disse's spaces widened. Occasional foci of a few polymorphonuclear leukocytes marked these loci. The portal fields contained some round cells, and with the exception of the arteries the structures were not remarkable. The arteries and the arterioles were edematous and the muscle nuclei showed the changes already described in the vascular myocytes of the other organs.

The lymph node removed from the right main bronchus was a caseous mass of necrotic tissue surrounded by a zone of fibrosis and characterized by the presence of giant cell tubercles. Nodes from the mesentery showed reticuloendothelial hyperplasia of the same type as that noted in the spleen, and a minimal amount of erythrophagocytosis.

*Pathologic Diagnosis*—Generalized arteritis, acute thromboarthritis, interstitial pneumonia, interstitial nephritis, generalized acute lymphadenitis, tuberculous bronchial lymphadenitis, chronic suppurating abscess of a calix of the left kidney, calculus of the left ureter, mild hydropnephrosis of the left kidney, benign hypertrophy of the prostate gland.

**CASE 3**—A 64 year old white woman was admitted to the Medical College of Virginia Hospital Jan 24, 1944 because of a psychosis. During the previous year she had suffered from a persistent cutaneous rash said to have been caused by sensitivity to soap, she was treated for this by numerous physicians, without success. Because of a small burn of the right gluteal region, sulfanilamide dressings were applied January 29. Up to this date the patient was organically well except for hypertension (170 systolic and 110 diastolic). On January 30 the oral temperature was found to have risen to 101.3 F, and sulfadiazine therapy was consequently instituted, a total of 350 grains (22.5 Gm) being administered during the following four days. By February 1 the oral temperature had risen to 104 F and remained at about that level until February 6, when the drug was discontinued. On February 4 a generalized maculopapular rash spread over the patient's body. The clinical interpretation of her condition at this time was pneumonia. Since the sulfadiazine therapy appeared to have little beneficial effect, it was discontinued, however, the sulfanilamide dressings were not but were applied until the patient's death, February 8.

On her admission to the hospital, her urine had shown no abnormalities. On the day of her death it contained albumin (2 plus), 4 to 6 leukocytes per high power field and many granular casts. Unfortunately, no studies of renal function were undertaken except for a determination of the nonprotein nitrogen of the blood on admission, 29 mg per hundred cubic centimeters. The sulfadiazine levels on February 3 and 5 were, respectively, 176 and 186 mg per hundred cubic centimeters. A hematologic study on February 3 revealed 3,800,000 erythrocytes and 12,500 leukocytes per cubic millimeter. The differential count was 91 per cent neutrophilic polymorphonuclear leukocytes, 5 per cent lymphocytes and 4 per cent eosinophilic leukocytes.

*Gross Examination*—A maculopetechial rash covered the entire body. The efflorescence was confluent on the backs of the hands, the elbows and the thighs. A recent second degree burn, 8 cm in the longest dimension, was present in the right gluteal region.

The left pleural cavity contained 600 cc and the right 500 cc of clear straw-colored fluid.

The right and the left lung weighed respectively 725 and 500 Gm. The pleural cavity of the right hemithorax was largely obliterated by fibrous adhesions. The lung was firm and subcrepitant throughout. On section the blood could be expressed with ease, no areas of consolidation were visible. The left lung was crepitant throughout except for several small areas in the lower lobe.

Each kidney weighed 150 Gm. The capsules stripped with difficulty, revealing scarred surfaces. On bisection of the organs, the cortices appeared narrowed, but the structures were essentially not remarkable.

*Microscopic Examination*—The myocardial interstices were occupied by a cellular exudate composed of monocytes, some neutrophilic and eosinophilic polymorphonuclear leukocytes and histiocytic cells possessing a large amount of cytoplasm, some showing active erythrophagocytosis. The exudate was particularly rich about vessels. The myocytes were fragmented.

Numerous sections from both lungs revealed foci of consolidation about bronchioles. The alveoli contained an exudate composed of neutrophilic leukocytes, monocytes and basophilic nondescript debris and fibrin. In these areas the bronchiolar epithelium was desquamated. Two sections showed marked alveolar capillary congestion. Within the septums were numerous leukocytes similar in type and distribution to those described in the heart. Many of the alveoli were lined with fibrinous membranes. Conglutinated thrombi filled some of the interalveolar capillaries.

In the kidneys there were foci of cortical fibrosis wherein the glomeruli were completely fibrosed and hyalinized. A dense round cell exudate was present in these areas. Aside from these changes the glomeruli were not remarkable; however, the epithelium of the convoluted tubules showed cloudy swelling, far advanced in some foci. In these areas small collections of monocytes were present. The arteries showed intimal fibrosis and thickening.

Focal cellular exudates consisting of monocytes and neutrophilic leukocytes obscured the acinous structure of the pancreas. The interstices were edematous and the vessels were surrounded by cellular mantles similar in composition to those seen in the parenchymal lobules. The media of small arteries was thickened and the myocytic structure obscured by fibrinoid necrosis. The nuclei of the muscle cells were distorted and pyknotic, and many of the arterioles were filled with conglutinated thrombi.

The hepatic portal fields were prominent because of a heavy monocyctic exudate, among which were neutrophilic and eosinophilic leukocytes as well as plasmotoid cells. In some areas the connective tissue matrix stained the uniform deep red of fibrinoid necrosis. Occasional small hepatic arteries showed changes similar to those seen in the pancreas (fig 7). Kupffer cells were easily visualized because of wide Disse's spaces. The hepatic cords were intact, but cloudy swelling and fat vacuoles were seen throughout the organ.

The splenic follicles were hyperplastic, an eosinophilic homogeneous substance was present in many germinal centers. The pulp was congested, and numerous eosinophilic and neutrophilic leukocytes were present.

*Pathologic Diagnosis*—Generalized arteritis, interstitial myocarditis, serous hepatitis, acute interstitial pancreatitis, interstitial and focal pneumonia, bilateral hydrothorax, fibrous pleural adhesions, generalized atherosclerosis.

*CASE 4*—On June 6, 1944 a 53 year old white woman suddenly and without prodrome began to suffer from headache, chills and fever. She was treated by her local physician with tablets not believed to contain any sulfonamide compound. Because her condition grew steadily worse, a second physician was consulted. He administered 30 grains (1.92 Gm) of sulfathiazole in two doses and then, suspecting the possibility of Rocky Mountain spotted fever because of the appearance of an erythematous macular rash, sent her to the Medical College of Virginia Hospital. On admission the patient's temperature was 105 F. The urine revealed albumin (1 plus) and 10 to 18 leukocytes and 100 erythrocytes per high power field. A blood count showed 4,200,000 erythrocytes and 18,200 leukocytes per cubic millimeter. Although it was believed that Rocky Mountain spotted fever was the most plausible diagnosis, blood taken for agglutination tests on January 15 (nine days after the onset of the disease) was reported as showing no agglutinins for *Proteus* X-19 or X-2 or for organisms of the *Eberthella* and *Salmonella* groups. On admission the patient had been given 15 grams (0.96 Gm) of sulfacetimide (paraaminobenzene sulfonylacetylumide) by mouth and 60 grams (3.84 Gm) of sulfadiazine intravenously, a further dose of 15 grams (0.96 Gm) of sulfathiazole was administered by mouth June 12, 1944. Because it was believed that sulfonamide compounds were ineffective against rickettsial diseases, their use was discontinued. The patient showed no response to therapy, and on June 14 a second blood count showed relative leukopenia (5,800 leukocytes per cubic millimeter). On June 16 generalized edema was observed, the patient died in uremia on June 18.

*Gross Examination*—The petechial rash was present in the greatest concentration on the skin of the upper part of the abdomen and the backs of the hands and was scattered over the remainder of the body. The hands and feet showed pitting edema. In the axillary and inguinal regions the lymph nodes could be readily palpated. Each pleural cavity contained about 100 cc of clear yellow fluid.

The spleen was bound to the diaphragm and the lateral posterior abdominal wall by fine fibrous bands. The organ's consistency was moderately reduced, and on section the surface was gray-red. The parenchyma on section bulged and could be scraped away with ease.

The kidneys weighed, left, 275 Gm, right, 150 Gm. When the adipose and fibrous capsules were stripped, pale surfaces were revealed, and a few small cysts filled with clear fluid were visible on the surfaces of both organs.



The consistency of the liver was reduced, and on incision the cut surfaces were yellow-brown and greasy. The central venules were prominent, and the usual lobular markings were obscured.

The axillary, inguinal, para-aortic and mesenteric lymph nodes were enlarged, and when sectioned they revealed homogeneous pale brown surfaces.

*Microscopic Examination*—The myocardial fibers were fragmented, and the interstices were characterized by an exudate of histiocytic monocytes, plasma cells

the cells about the central venules. The portal spaces were infiltrated by numerous monocytes and a few neutrophilic polymorphonuclear leukocytes. Eosinophilic homogenization of the walls of small arteries was visible in some of the portal fields. The nuclei of the myocytes of these vessels showed distortion and karyorrhexis. The hepatic veins contained many liver cells showing all stages of degeneration.

The interstices of the pancreas were edematous and sparsely infiltrated by monocytes. The islets of Langerhans were not remarkable.

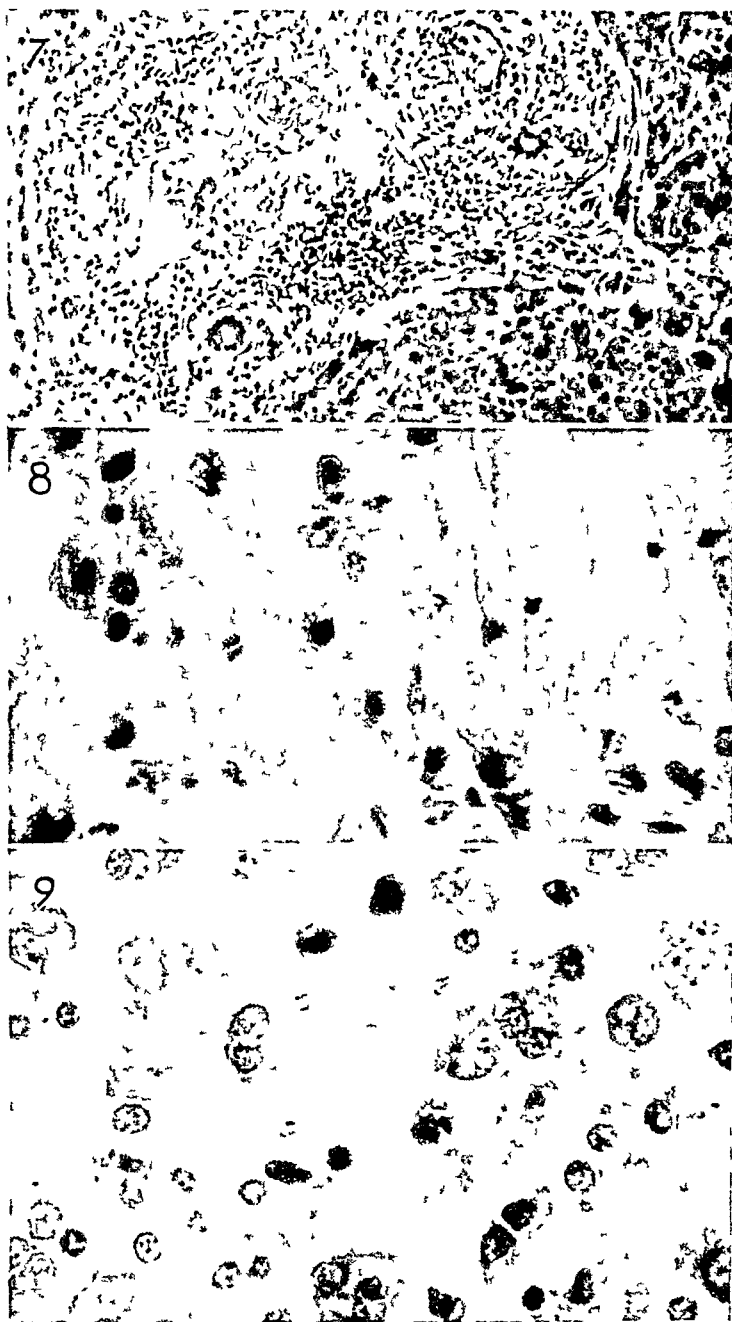


Fig 7 (case 3)—Portal field of the liver infiltrated by round cells, fibrinoid necrosis of a small hepatic artery ( $\times 135$ )

Fig 8 (case 4)—Characteristic histiocytic interstitial myocarditis ( $\times 540$ )

Fig 9 (case 4)—Sinusoid of a lymph node filled with proliferated reticuloendothelial cells, many are frank macrophages containing erythrocytes ( $\times 765$ )

and very occasional neutrophilic polymorphonuclear leukocytes (fig 8). The exudate was most prominent in the inner third of the myocardium and the zone adjacent to the epicardium.

The hepatic structure was disorganized by marked fragmentation of the trabeculae and early necrosis of

Both kidneys presented typical interstitial infiltrates of monocytes, neutrophilic polymorphonuclear leukocytes, lymphocytes and plasma cells. These exudates were thickest about glomeruli and small vessels. The glomerular loops contained little blood, many tufts were bloodless, and precipitated protein substance lay within



**Bowman's spaces** The convoluted tubules were bright pink and showed marked cloudy swelling, in some instances the nuclei were undergoing karyorrhexis. Many of the tubules contained hemoglobin casts, within which erythrocytes were still recognizable. No crystals were seen. The perirenal and peripelvic connective and adipose tissues were infiltrated by inflammatory cells of the same type as those described in the parenchyma. An occasional segment of the wall of a small artery was converted into homogeneous fibrinoid material.

Large zones of ischemic necrosis were observed in the spleen. Within these areas the sinusoids contained only erythrocytic shadows, connective tissue trabeculae were indistinct, and all nuclei were pyknotic or broken up into small particles, giving a blue cast to the necrotic zones. In the viable regions lymphoid follicles were hyperplastic and showed various stages of central necrosis. The intima of the pencil arteries was thickened and eosinophilic, and medial nuclei were undergoing early karyorrhexis. The subendothelium was infiltrated by monocytes, including occasional polymorphonuclear leukocytes. The reticuloendothelial cells were prominent. The sinusoids of both the medulla and the cortex were filled with large mononuclear cells, many showing phagocytosis of erythrocytes and leukocytes. Nowhere, either leading to or in the regions of necrosis, could obstruction of vessels be demonstrated.

Histologic study of lymph nodes taken from various sites showed a more or less uniform appearance. There was marked reticuloendothelial hyperplasia, resulting in a filling of all the sinusoids with cells similar in all respects to those described in the spleen. The origin of these cells is demonstrated by their ability to phagocytose erythrocytes, lymphocytes and a small amount of hemosiderin (fig. 9). Lymphoid follicles were discernible only by their hyperplastic germinal centers. The capsules and the surrounding connective tissue were infiltrated by monocytes, plasmotoid cells and occasional polymorphonuclear leukocytes both neutrophilic and eosinophilic.

Sections of sternum, rib and vertebral marrow revealed large reticuloendothelial cells of the same type as those seen in both the spleen and the lymph nodes. They showed much phagocytosis of erythrocytes and leukocytes. No necrosis was seen.

The uterine myometrium was not remarkable, the endometrial glands were arranged in a typical proliferative pattern. The small uterine arteries showed homogeneous eosinophilic thickening of their intima.

The stratified squamous epithelium of the esophagus was represented by small islands showing essentially the normal structure. Between these areas of intact epithelium the surface was denuded of its lining. The submucosa was massively infiltrated by neutrophilic polymorphonuclear leukocytes, monocytes and occasional plasmotoid cells. The small arteries throughout the organ were characterized by vacuolation of the muscularis and distortion of the nuclei, many of which showed early karyorrhexis. Some were the site of typical foci of fibrinoid necrosis surrounded by monocytes, plasma cells and eosinophilic leukocytes. The smooth musculature of the organ showed fragmentation and nuclear pyknosis. The mediastinal connective tissue was edematous and infiltrated by inflammatory cells of the same type as those described about the vessels.

A small area of mucosa in the ileum was absent, and the base of the shallow ulcer was hemorrhagic. The small arteries showed typical fibrinoid necrosis and again the characteristic monocytic exudate with

its erythrophagocytic histiocytes was prominent, some of the vessels were filled with conglutinated thrombi.

Numerous sections of skin taken at various sites through the maculas contained small arteries in which the media was vacuolated. Some of the nuclei were absent, others were distorted and showed marked tinctorial abnormalities interpreted as early degeneration. Some of the vessel walls were homogeneous, characteristic of fibrinoid necrosis, and surrounded by the thin mantle of monocytes so frequently noted in other organs. The walls of the necrotic vessels were likewise infiltrated by the leukocytes. Many of these arteries were surrounded by extravasated erythrocytes. The entire subcutaneous fat was diffusely infiltrated by cells of the same type, some of which showed phagocytic activity. The dermis subjacent to the epithelium was edematous and similarly infiltrated.

**Pathologic Diagnosis**—Generalized arteritis, interstitial myocarditis, interstitial nephritis, serous hepatitis, focal splenic necrosis (Feitis), ulcerating esophagitis and ileitis, generalized acute lymphadenitis.

**CASE 5**—In this case, Dr. William Branch Porter as consultant recognized the syndrome, and Dr. James W. Eliot submitted the material to the department of pathology of the Medical College of Virginia.

A 51 year old white woman was treated, May 1, 1943, for acute pharyngitis with 45 grains (2.88 Gm.) of sulfadiazine daily. May 3, 1943, she was admitted to a hospital, where the therapy was continued. May 4, 22.5 grains (1.44 Gm.) of sulfanilamide was given, followed on May 5 and 6 by 37.5 grains (2.4 Gm.) of the same drug. About May 7 she "broke out over her chest, abdomen, back, and hips with urticarial wheals, one-half to one and one-half inches in diameter, white in the center and red at the periphery." The administration of sulfanilamide was thereupon suspended, but that of sulfadiazine was continued. The rash disappeared within "two or three days," and on May 13 treatment with sulfadiazine was also discontinued. She received no sulfonamide drug for the next eight days, then (May 21) 90 grains of sulfanilamide (5.76 Gm.) and 20 cc of azosulfamide were given, the latter by intramuscular injection. About 25 cc of azosulfamide was regularly administered until May 31, when all medication with sulfonamide drugs was again suspended. May 28, 135 grains (8.64 Gm.) of sulfanilamide was given, followed by a similar dose the next day. A disseminated rash appeared on the succeeding day (May 30) accompanied by congestion of the conjunctivas and a mildly icteric appearance of the scleras. Because renal function showed progressive impairment, with the blood urea nitrogen rising to 43 mg. per hundred cubic centimeters, a medical consultation was sought. The patient was seen by the consultant about June 1, at that time she was still covered by a maculopapular rash. Although the administration of sulfonamide compounds was immediately stopped and measures taken to stimulate renal function, the patient died June 4 in uremia.

The heart and blocks of lung, liver, spleen and gallbladder were received in formaldehyde.

**Microscopic Examination**—The myocardial fibers were separated by edema, within the interstitial spaces was a diffuse exudate of histiocytes and plasmotoid cells, whose cytoplasm was markedly eosinophilic. Among these cells were some lymphocytes and occasional polymorphonuclear leukocytes. Phagocytosis of leukocytes was seen in some foci.

The parenchymal cords of the hepatic lobules were fragmented. In the central zones, the nuclei showed

early karyorrhexis, and the cell cytoplasm contained numerous vacuoles. Sinusoids were dilated, and Kupffer cells were prominent and vacuolated. Disse's spaces were distended, and a moderate number of polymorphonuclear leukocytes were scattered throughout the sinusoids and the portal fields.

Renal convoluted tubules showed degenerative changes running the gamut from marked cloudy swelling to necrosis, the latter being sharply focal, within these areas the cellular outlines and nuclei could no longer be seen. The lumens were filled with acidophilic material probably derived from desquamated necrotic epithelium. Hyaline casts were numerous and appeared in all segments of the nephrons. The glomerular capillaries contained few erythrocytes and their basement membranes were thickened.

The peribronchial alveoli were filled with an exudate rich in monocytes, polymorphonuclear leukocytes and fibrin. The adjacent bronchioles contained purulent exudate and their walls were infiltrated by both polymorphonuclear leukocytes and mononuclear cells.

Splenic follicles were not prominent; the sinusoids were packed with erythrocytes and the endothelial cells were hyperplastic.

The small arteries in all organs examined were characterized by edema of the media and swelling of the intima. Some of the muscle nuclei showed structural changes indicative of nuclear damage.

*Pathologic Diagnosis*—Interstitial myocarditis, necrosis of renal tubules (nephrosis), serous hepatitis, focal pneumonia.

#### COMMENT

In general the tissues in the 5 cases were characterized by two related lesions: arterial changes (except in case 1) and generalized cellular exudate.

The arterial lesions differed in appearance yet all were reflections of a linear progression from simple edema (which Rich<sup>19</sup> aptly compared to the edema of a cutaneous wheal) to frank necrosis. The nuclei of the myocytes underwent pyknosis or karyorrhexis and a circumferential segment of the arterial wall lost its cellular detail and assumed the eosinophilia of fibrinoid necrosis (fig. 4). The earliest cellular components of the exudate, neutrophilic leukocytes and lymphocytes appeared in the intima. Thereafter a moderate periadventitial exudate of lymphocytes, monocytes (some actively phagocytic), plasma cells and occasional eosinophilic and neutrophilic leukocytes made its appearance. The surrounding connective tissue matrix of some vessels underwent fibrinoid necrosis similar to that of the arteries themselves (fig. 4). Although none of the examples of necrotizing vascularitis seen was quantitatively comparable to the typical lesion of periarteritis nodosa, the basic pattern was so similar that it suggested a difference of intensity and duration of action rather than of quality of irritant.

The cellular exudate invading the connective tissue of the skin, the renal pelvis, the mediastinum, the gastrointestinal tract and other organs was similar in all respects to that mantling the smaller arteries. The most prominent component was a macrophage possessing a large amount of cytoplasm and displaying active phagocytosis not only of debris where this was available but of whole leukocytes and erythrocytes as well. Its nucleus was eccentrically situated and it sometimes resembled (in the hematoxylin and eosin preparation) a plasma cell so closely that only the presence of transitional types and the phenomenon of phagocytosis made differentiation possible.

The basic lesions of experimental protein anaphylaxis have been thoroughly investigated<sup>20</sup> and the overwhelming consensus is that the phenomenon is characterized by fibrinoid necrosis of both small arteries and connective tissue and the presence of a cellular exudate composed chiefly of monocytes, lymphocytes, plasma cells and eosinophilic and neutrophilic leukocytes. A decided tendency for the cells to arrange themselves perivascularly has likewise been recorded and the suggestion has been repeatedly made that periarteritis nodosa in man may represent an anaphylactic phenomenon.<sup>21</sup> Rich's<sup>19</sup> conclusions in this respect are the latest reaffirmation of this belief.

That the arterial lesions and the composition of the cellular exudate observed in the reactions following administration of sulfonamide compounds are similar in all respects to those of experimental anaphylaxis is apparent.

French and Weller,<sup>27</sup> examining the hearts of patients treated with sulfonamide drugs, noted an interstitial exudate containing large mononuclear cells of the "clasmatocytic type." All hearts of the present series showed interstitial myocarditis of this type. The cells of the exudate were similar to those observed by these two authors. The most prominent were macrophages of the same type as those described in the connective tissues. Phagocytosis of erythrocytes and leukocytes in the heart as seen in some of the cases of this series is rarely observed, and although the term "clasmatocyte" is generally applied to cells capable of phagocytosis, French and Weller do not record its observation.

Interstitial myocarditis is the lesion most consistently noted in experimental anaphy-

<sup>26</sup> Arthus<sup>2</sup>, Rich<sup>19</sup> and Gregory<sup>21</sup>, Henlein<sup>23a</sup>, Klinge<sup>23b</sup>, Vaubel<sup>23c</sup>, Apitz<sup>23d</sup>.

<sup>27</sup> French, A. J., and Weller, C. V. *Am. J. Path.* 18: 109, 1942.

laxis<sup>26</sup> Clark and Kaplan<sup>29</sup> and Rich<sup>19</sup> saw this change in their cases of human serum sickness, and it has been repeatedly described in instances of fatal reactions to sulfonamide compounds.

The presence of the lesion in all 5 cases of this series is confirmation, in man, of the experimental observation that interstitial myocarditis is one of the commoner, perhaps the most frequent, of the expressions of generalized anaphylaxis.

In 4 of the cases the characteristic vascular lesions were readily seen in the heart (fig. 2). While the myocardial fibers showed marked degeneration in the areas of greatest cellular exudation foci of necrosis such as those described by Lederer and Rosenblatt were not encountered.

Multiple ischemic infarction of the spleen was first described by Feitis in 1921. Since then approximately 29 cases have been reported<sup>30</sup> in most of which the infarcts were caused by thrombosis of splenic arteries. However, several cases without vascular obstruction have been recorded. It is believed that cases 1 and 4 belong in the latter category. The infarcts involved not merely the follicles but the red pulp as well, and nowhere was occlusion of vessels demonstrable, although the small arteries in case 4 showed marked fibrinoid necrosis.

Explanations of this phenomenon are not easily formulated. Enzer<sup>31</sup> drew a parallel between Mallory's theory of hepatic sinusoidal obstruction by macrophages (by means of which Mallory explained the typical typhoid lesions of the liver) and the presence of numerous macrophages within the perifollicular sinusoids of the spleen in his case. Magnus<sup>32</sup> who described a case of multiple splenic infarction without visible vascular occlusions, expressed the belief that some toxic factor may have injured the tissue, producing the necrosis.

It appears significant that in 2 of 5 cases in this small series the spleen showed a lesion which is so uncommon that up to 1938 only 29 instances had been recorded in the literature.

Numerous authors<sup>33</sup> have reported focal follicular necrosis of the spleen and the lymph nodes and a similar lesion of marrow in reactions

to sulfonamide drugs. Necrosis of lymph nodes was observed in 2 of the cases of serum sickness studied by Rich<sup>19</sup> (cases 17250 and 12291). In the present series such lesions of the lymph nodes were not seen.

Phagocytosis was most prominent in these same areas (fig. 9). The reticuloendothelial system is largely concentrated in the spleen, the lymph nodes and the bone marrow, and since it gives rise to both macrophages and antibodies (including opsonins which mediate phagocytosis) it is not surprising that in a state of generalized hypersensitivity, phagocytosis of cellular debris should be active and readily noted near foci of necrosis in these organs.

The same phenomenon, involving whole erythrocytes and leukocytes in distant, non-necrotic areas is less easily explained. To assume arbitrarily that these corpuscles are physically damaged does not appear warranted. Neither does the anthropomorphic concept of a "reticuloendothelial system gone wild" offer a sounder explanation. Much more consonant with present day concepts may be the theory that the leukophagocytosis and the erythrophagocytosis represent a form of "foreign body reaction" in which the homologous blood cells are so changed by the addition of a substance (perhaps a conjugated sulfonamide group) as to render them alien to the protein pattern of the patient. Such a change may conceivably convert the erythrocytes or the leukocytes into allergens capable of eliciting the production of specific antibodies. Dameshek,<sup>34</sup> indeed, suggested that this mechanism was the probable method of production of the "cold hemagglutinins" which are a factor in the hemolytic anemia that sometimes follows treatment with sulfonamide compounds.

It appears that agranulocytosis and leukopenia, both of which may complicate the therapeutic use of sulfonamide compounds, may represent the analogue of hemolytic anemia. This concept finds support in experimental literature as well as in clinical experience. Chew<sup>35</sup> and co-workers produced in guinea pigs by means of leukotoxic serums not merely marked leukopenia but definite neutropenic states comparable to agranulocytosis. The antibodies causing neutrophils to disappear from the blood were obtained in exactly the same manner in which hemolysins are ordinarily produced by inoculating leukocytes into an animal of a "foreign" species (rabbit).

28 Longcope W T J Exper Med **22** 793, 1915  
Hemlein<sup>23a</sup> Klinge<sup>23b</sup> Vaubel<sup>23c</sup> Apitz<sup>23d</sup>

29 Clark, E, and Kaplan, B J Arch Path **24** 458, 1937

30 Schmeisser, H C, and Harris, L C Am J Path **14** 821, 1938

31 Enzer, N Am J Path **2** 511, 1926

32 Magnus H A J Path & Bact **44** 103, 1937

33 Lederer and Rosenblatt<sup>17</sup> Meikel and Crawford<sup>18</sup> Rich<sup>19</sup>

34 Dameshek, W J A M A **123** 77, 1943

35 Chew, W B, Stephens, D J, and Lawrence, J S J Immunol **30** 301, 1936

Dameshek and Colmes<sup>36</sup> were able to reproduce agranulocytosis with minimal doses of aminopyrine (5 mg in their case 1) in persons in whom the condition had previously developed during medication with aminopyrine. Furthermore, they obtained positive intradermal reactions to aminopyrine incubated with human serum (conjugation) after failing with aqueous solutions of the pure chemical. Many reports of cases in which agranulocytosis followed treatment with sulfonamide compounds<sup>37</sup> demonstrate that in previously untreated persons the lesion appears shortly after the inception of the syndrome of serum sickness, when antibodies first become demonstrable experimentally.

It is certainly not inconsistent with the facts to state that the concepts of direct toxic chemical depression of marrow activity and "inhibition of maturation"<sup>38</sup> are at the very least, open to objections just as grave as any that may be directed against the anaphylactic concept of agranulocytosis. The combination of the two factors (anaphylaxis and selective chemical toxicity of the marrow) presents a more satisfactory approach to the problem of both leukopenia and agranulocytosis.

In the 5 cases the liver showed little individual variation. In no instance was widespread necrosis such as characterizes acute yellow atrophy seen nor, for that matter, the aseptic necrobiotic foci said to be especially numerous in this organ in reactions to sulfonamide compounds. On the other hand all the changes described by Rossle and Eppinger<sup>39</sup> as peculiar to serous hepatitis were demonstrable (fig 1). The parenchymal trabeculae in most cases were fragmented, the cell cytoplasm was granular. Disse's spaces and Kupffer cells were prominent. Present within the sinusoids and portal fields was an exudate consisting of the same cellular elements so often described in other sites. Within some of the portal fields, small hepatic arteries reflected the generalized vasculitis, and the connective tissue showed the fibrinoid necrosis so typical of reactions to sulfonamide drugs.

Rossle and Eppinger in the course of their separate investigations of hepatic disease described what they called (hepatosis) serous hepa-

titis. The lesion in its earliest form is similar to that seen in case 3. The chief morphologic change is the appearance of the normally invisible Disse's spaces, which lie between the endothelium of the sinusoids and the liver cells. The phanerosis of these spaces is said to be due to the circumstance that fluid has been lost from the sinusoids because of increased permeability of their lining membrane as in any inflammatory lesion, and has accumulated within the spaces. This interferes with the nutrition of the cells, and if the process is not reversed, the liver cords dissociate (case 5) sometimes producing jaundice. Should the process continue hepatic necrosis, varying from foci to acute yellow atrophy, may appear. Serous hepatitis is not a pathognomonic lesion but an expression of increased vascular permeability; in this respect the hepatic changes may be likened (just as the early arterial lesions were) to the edematous wheal of cutaneous allergy.

Observations on the kidneys occupy a prominent position in the literature of fatal reactions to sulfonamide drugs since progressive uremia is so frequent a symptom. In 3 cases of the present series the kidneys showed typical focal interstitial round cell exudates, avascular glomeruli, interstitial edema and marked cloudy swelling of the tubular epithelium. In addition to these lesions the kidneys in case 2 showed isolated convoluted tubules that were necrotic (fig 5) and some surrounded by a scant neutrophilic exudate.

Multiple foci of well advanced necrosis of the convoluted tubules unaccompanied by interstitial exudate were seen in case 5. The remaining epithelium was swollen, granular and obviously on its way to sharing the fate of that at the sites of the more advanced lesions. This is the only unequivocal example of nephrosis in the series—a reversal of the usual ratio of nephrosis to interstitial nephritis in previous reports.

Kimmelstiel<sup>40</sup> comprehensively reviewed the literature of human interstitial nephritis. One of his conclusions was "interstitial nephritis is regarded as an allergic hyperergic reaction to foreign proteins or protein split products." Certainly interstitial nephritis is a common renal lesion in experimental anaphylaxis.<sup>41</sup>

That the nephrosis so frequently reported in reactions to sulfonamide drugs may represent a form of hyperergia and not direct chemical irritation is indicated by the fact that renal

36 Dameshek, W., and Colmes, A. *J. Clin. Invest.* **15** 85, 1936.

37 Sheket, H. A., and Price, A. E. *J. A. M. A.* **112** 823, 1939. Briggs, G. O. A. *Lancet* **2** 739, 1939. Arrowsmith, W. R., Binkley, B., and Moore, C. V. *Ann. Int. Med.* **21** 323, 1944.

38 Fitz-Hugh, T., Jr., and Krumbhaar, E. B. *Am. J. M. Sc.* **188** 104, 1932.

39 Rossle and Eppinger, cited by Eppinger, H. *Die Leberkrankheiten*, Berlin, Julius Springer, 1937.

40 Kimmelstiel, P. *Am. J. Path.* **14** 737, 1938.

41 Longcope, W. T. *J. Exptl. Med.* **18** 678, 1913. Heinlein<sup>23a</sup>, Klinge<sup>23b</sup>, Vaubel<sup>23c</sup>.

involvement frequently appears synchronously with, or following, the cutaneous rash (case 5), evidence of antigen-antibody reaction in other tissues. Furthermore, in case 5 uremia manifested itself only during the second reaction at a time when it may be surmised that sensitivity to sulfamylamide had increased. In many instances of nephrosis demonstrated to be due to therapy with a sulfonamide compound the amount of drug used was so small as to exclude as unreasonable any explanation other than that of anaphylaxis.

Rackemann, Longcope and Peters<sup>42</sup> investigated the clinical significance of the edema of serum sickness and concluded that it as well as oliguria (which, they stated may progress to urinary suppression) is due to damage of the kidney. The nature of the lesion was not investigated.

The pancreatitis in case 3 is an unusual lesion. Rich<sup>20</sup> mentioned without further comment a similar pancreatitis in a case in his series. The cause is suggested in the focal fibrinoid necrosis of the small arteries and in the identity of the exudate with that demonstrated at other sites. Nonhemorrhagic interstitial pancreatitis is an unsolved problem, certainly the lesions in case 3 and in the case of Rich indicate that one of the etiologic mechanisms may be anaphylaxis.

Rich<sup>20</sup> described the underlying pulmonary lesion in the same case in which the pancreatitis was observed as capillitis with interalveolar exudation. Rich and Gregory<sup>43</sup> and also Pinkerton<sup>44</sup> elaborated on the appearance of this peculiar pneumonitis emphasizing the vascular necrosis and the subsequent interalveolar infiltration as well as the appearance of a fibrin membrane outlining the alveolar walls. Where the stimulation was sufficiently powerful, necrosis occurred. Gessler<sup>24</sup> noted the same lesion in his case 1.

The lungs in my cases 2 and 3 showed similar lesions (fig 3). Rich and Gregory,<sup>43</sup> furthermore, compared the pneumonitis which they observed to that encountered in 5 patients with rheumatic heart disease, the processes were identical, and since the pulmonary lesions following administration of sulfonamide compounds are the reflection of a pulmonary anaphylactic reaction they concluded that this form of rheumatic pneumonia was added evidence of the allergic nature of rheumatic fever.

The experimental work of Cannon, Walsh and Marshall<sup>45</sup> resulted in the production of anaphylactic pneumonia, which was described as acute pneumonitis characterized by edema, alveolitis, bronchitis and focal pneumonic consolidation. Acute arteritis and phlebitis occurred at times and mural thrombosis was occasionally encountered.

While convincing experimental evidence of the morphologic nature of anaphylactic pneumonia is lacking the evidence at hand would indicate that it is essentially an interstitial process (interstitial pneumonitis).

In case 2 the aorta presented a unique lesion. Acute thromboarthritis produced in man in the course of reactions to sulfonamide compounds has never been described. Lehr and Antopol<sup>46</sup> did observe medial necrosis and calcification of the thoracic aorta in rats given a large single dose of sodium sulfadiazine. Junghans (quoted by Heinlein<sup>27a</sup>) examined the aortas of rabbits sensitized to hog serum and found in each mucoid degeneration of the thickened intima which was infiltrated by monocytes up to and including the innermost medial muscle fibers.

Seegal, Seegal and Jost,<sup>47</sup> using rabbits, produced a local Arthus reaction within the pericardial sac. They observed intimal thickening and subendothelial polymorphonuclear leukocytic exudate of the aorta.

The thromboarthritis in case 2 represented in its pattern the same type of reaction as the process seen in the smaller arteries.

Available evidence does not permit any conclusions concerning the nature of the lesion. The suggestion is advanced, however, that in view of the generalized nature of the reaction that sometimes follows treatment with sulfonamide compounds and the fact that Junghans did demonstrate hyperergic aortitis in rabbits, the thromboarthritis may be the result of an anaphylactic reaction.

It is obvious that more questions are suggested than are answered by the preceding exposition, however, the protean nature of generalized anaphylaxis is indicated.

The sulfonamide drugs were administered in all cases except case 2 for some underlying disease, which may have contributed to the lesions observed. This possibility should not lightly be brushed aside, yet numerous authors have inde-

42 Rackemann, F. M., Longcope, W. T., and Peters, J. P. *Arch. Int. Med.* **18**: 496, 1916.

43 Rich, A. R., and Gregory, J. E. *Bull. Johns Hopkins Hosp.* **73**: 465, 1943.

44 Pinkerton, H. J. *Missouri M. A.* **40**: 364, 1943.

45 Cannon, P. R., Walsh, T. E., and Marshall, C. E. *Am. J. Path.* **17**: 777, 1941.

46 Lehr, D., and Antopol, W. *Urol. & Cutan. Rev.* **45**: 545, 1941.

47 Seegal, D., Seegal, B. C., and Jost, E. L. *J. Exper. Med.* **55**: 155, 1932.

pendently recorded the same lesions in various unrelated disease states in which the only common factor was the administration of sulfonamide compounds

The identity of the lesions, with those produced in a few human subjects and many animals by known species-foreign proteins the knowledge that the sulfonamide compounds may convert homologous proteins into allergens and the fact that characteristic clinical syndromes follow the administration of the drugs constitute sufficient evidence to justify the concept of the anaphylactic nature of the lesions described

#### SUMMARY

The relatively large number of deaths accompanying the universal use of sulfonamide drugs has made possible for the first time a systematic study of progressive fatal human anaphylaxis from the standpoint of pathology

The basic lesion, as in experimental protein anaphylaxis is necrotizing fibrinoid arteritis of the smaller vessels. The cellular exudate is monocytic in composition. The reticuloendothelial system is hyperplastic, in the spleen the lymph nodes and the marrow the sinusoids may be crowded with macrophages showing phagocytosis of erythrocytes and leukocytes.

It is suggested that this phenomenon may be the morphologic expression of the addition of some substance to the blood cells (conjugated sulfonamide group?) that renders them "foreign." The possibility that these homologous "foreign cells" may elicit the production of antibodies is suggested and may account for the hemolytic anemia, the leukopenia and the agranulocytosis.

The available evidence indicates it is believed, that the nephrosis in cases of reaction to treatment with sulfonamide compounds is a form of anaphylactic renal reaction.

## PRIMARY SYSTEMIC AMYLOIDOSIS

STUART LINDSAY, M.D., AND WILLIAM F. KNORP, M.D.

SAN FRANCISCO

SAN MATEO, CALIF

Primary systemic amyloidosis is a rare disease of unknown cause. It differs from the more common secondary amyloidosis in several respects: (a) the absence of specific etiologic factors such as tuberculosis or chronic suppuration, (b) the minimal deposition of amyloid in the liver, the spleen, the kidneys and the adrenal glands, the sites of maximum deposition in the secondary type of amyloid disease, (c) the maximum deposition of amyloid in the heart, the lungs, the skin, the mucous membranes and other tissues not usually involved in the secondary type, (d) the occasional occurrence of amyloid tumors, (e) the atypical reactions with specific amyloid stains.<sup>1</sup>

To date 39 cases of primary systemic amyloidosis have been recorded. Koletsky and Stecher<sup>2</sup> collected 23 cases reported in the literature up to 1939, and added a case of their own. Their report included an extensive tabulation of the clinical and pathologic changes noted in these cases. Additional reports published before 1939 include those of Beneke and Bonning,<sup>3</sup> Silwer and Lindblom,<sup>4</sup> Larsen,<sup>5</sup> Koller,<sup>6</sup> Israel,<sup>7</sup> Gerber,<sup>8</sup> and Kerwin.<sup>9</sup> Since 1939 cases have been reported by Binford,<sup>10</sup> Pearson, Rice and Dickens,<sup>11</sup> Sappington, Davie and Horneff,<sup>12</sup>

and Dillon and Evans.<sup>13</sup> These additional cases, together with our own case are summarized in the accompanying table.

The additional example of primary systemic amyloidosis which we record presented an unusual combination of pathologic changes. The diagnosis was not made until after death although biopsy specimens from the external ear and the nasal mucosa both contained unrecognized deposits of amyloid.

### REPORT OF A CASE

P. B., a 56 year old Frenchman entered the Mills Memorial Hospital in San Mateo, Calif., Oct. 9, 1936, complaining of redness, swelling and pain over the right thenar eminence following a superficial abrasion of the skin. An abscess was incised and drained, with the patient under general anesthesia. His temperature on entry was 104 F. It promptly returned to normal after the operation. The postoperative course was uneventful.

He entered the hospital again on Jan. 22, 1941, with a three day history of pain in and purulent drainage from the right ear. Sagging of the posterior wall of the right external acoustic meatus and perforation of the right tympanic membrane were noted. A roentgenogram showed mastoiditis on the right side.

At this time the hemoglobin was 90 per cent (13.05 Gm.), the red cell count 4,080,000 and the white cell count 17,450, with polymorphonuclear leukocytes 40 per cent (nonsegmented 4 per cent), lymphocytes 59 per cent and monocytes 1 per cent. The urine was normal. January 24 the white blood cell count was 12,900, with polymorphonuclear leukocytes 37 per cent (7 per cent nonsegmented), large lymphocytes 3 per cent, small lymphocytes 58 per cent and monocytes 2 per cent.

Mastoidectomy was done on the right side and a large amount of purulent material and necrotic mastoid cells were encountered. His postoperative course was uneventful and afebrile. He was given sulfathiazole, 7 Gm. the first twenty-four hours and 4 Gm. every twenty-four hours for the next three days. The eustachian tubes were inflated on several occasions during the next two months.

The patient again entered the clinic, Jan. 19, 1942, with the complaints of (a) pain in the thoracic part of the spine, aggravated by movement, coughing or jarring, (b) loss of 15 pounds (6.5 Kg.), (c) bilateral nasal obstruction, (d) nasal bleeding of about 500 cc. per month and (e) epigastric pain beginning two hours after meals and relieved by alkalis taken before and

12 Sappington, S. W., Davie, J. H., and Horneff, J. A. *J. Lab. & Clin. Med.* **27**: 882, 1942.

13 Dillon, J. A., and Evans, L. R. *Ann. Int. Med.* **17**: 722, 1942.

From the Division of Pathology, University of California Medical School, San Francisco, and Mills Memorial Hospital, San Mateo.

1 Lubarsch, O. *Virchows Arch. f. path. Anat.* **271**: 867, 1929.

2 Koletsky, S., and Stecher, R. M. *Arch. Path.* **27**: 267, 1939.

3 Beneke, R., and Bonning, F. *Beitr. z. path. Anat. u. z. allg. Path.* **44**: 362, 1908, cited by Larsen<sup>5</sup> and by Binford<sup>10</sup>.

4 Silwer, H., and Lindblom, A. D. *Acta med. Scandinav.* **64**: 529, 1926.

5 Larsen, R. M. *Am. J. Path.* **6**: 147, 1930.

6 Koller, F. *Schweiz. med. Wchnschr.* **13**: 522, 1932.

7 Israel, I. *Ein Fall von lokalen Amyloid*, Med. Dissert., Tübingen, Bochum-Langendreer, 1933, cited by Koletsky and Stecher<sup>2</sup> and by Spain and Barrett<sup>20</sup>.

8 Gerber, I. E. *Arch. Path.* **17**: 620, 1934.

9 Kerwin, A. J. *J. Lab. & Clin. Med.* **22**: 255, 1936.

10 Binford, C. H. *Arch. Path.* **29**: 314, 1940.

11 Pearson, B., Rice, M. M., and Dickens, K. *LaV. Arch. Path.* **32**: 1, 1941.



Author	Year	Age	Sex	Symptoms	Clinical Diagnoses	Duration	Cause of Death	Distribution of Amyloid
Bencke and Bonning 4	1908	70	M	Not known	Bronchitis	Not known	Not known	Heart, vena cava, lungs
Silver and Lindholm 4	1926	34	M	Dyspnea, weakness, edema of legs, cyanosis, mental depression	Myocardial insufficiency	11 mo	Cardiac failure	Myocardium, mitral valve, arteries of heart, liver, spleen, kidneys and lungs
Iarsen 5	1930	65	M Negro	Cough, dyspnea, epigastria tenderness	Coronary arteriosclerosis with cardiac insufficiency	1 yr	Myocardial failure, streptococcal meningitis and septemia	Myocardium, endocardium, pulmonary artery, aorta, vena cava, lungs, kidneys, pararenal lymphon
Koller 6	1932	30	F	Dyspnea, edema of legs	Myocardial insufficiency, coronary sclerosis and diabetes	4 mo	Cardiac failure	Mitral and tricuspid valves, vena cava, tongue, myocardium, arteries of lungs, heart, liver, pancreas and bone marrow
Israel 7	1933	-	-	Data not available	Data not available	-	-	Mitral, tricuspid and pulmonary valves, heart, mouth, larynx, skin, mediastinum
Gerber 8	1934	44	M	Pruritus, postprandial pyrosis, vague muscular pains, weakness	Amyloidosis, amyloid nephrosis, hypercholesteremia, lipid histiocytosis	2 yr	Asotemia pulmonary edema	Diffuse amyloidosis of marrow, amyloidosis of kidney, liver, spleen, pancreas, stomach, thyroid and adrenal glands
Kerwin 9	1931	51	F	Fatigue, weakness, loss of weight	Chronic degenerative myocarditis	1 yr	Cardiac failure	Cardiac valves, myocardium, endocardium, pleura, pericardium, lung, stomach, large and small bowel, bladder
Kerwin 9	1936	39	F	Peripheral edema, dyspnea	Chronic degenerative myocarditis, myocardial insufficiency	4 mo	Cardiac failure	Myocardium aortic valves, pericardium, endocardium, liver, spleen, kidneys, adrenal glands
Binford 10	1940	56	M	Progressive dyspnea	Myocardial degeneration	10 mo	Myocardial failure	Myocardium, vessels of following lungs, liver, kidney, adrenal glands, bladder, prostate, seminal vesicles, spleen, pancreas, tracheobronchial lymph nodes, thyroid gland, diaphragm, femoral nerve
Pearson and others 11	1941	69	F Negro	Dyspnea, edema, papular lesions of mouth and tongue, purpura	Congestive heart failure	1 yr	Cardiac failure	Heart, lungs, esophagus, stomach, small intestine, spleen, vessels of thyroid gland
Pearson and others 11	1941	60	M Negro	Hoarseness, cough, hemoptysis, dyspnea, edema of ankles	Myocardial disease	10 mo	Irreversible obstructive	Pharynx, larynx, trachea, esophagus, stomach, diaphragm, subcutaneous tissues, peritrichial and peribronchial tissues, great vessels, auricles of heart, capsules of adrenal glands
Sappington and others 12	1942	51	F	Fatigue, dyspnea, distress in upper part of abdomen	Not stated	1 yr	Not stated, sudden death	Lungs, skin, systemic involvement of small and medium size arteries
Dillon and Evans 13	1942	61	M	Radiating substernal pain and dyspnea, loss of weight, purpura	Myocardial infarction, malignant lymphoma	6 mo	Cardiac failure	Liver, spleen, kidneys, blood vessels of all organs except spinal cord, including heart
Dillon and Evans 13	1942	57	F	Constipation, loss of weight, fatigue	Chronic nephritis amyloidosis, rheumatic heart disease, possible subacute bacterial endocarditis	2 yr	Hypertension, cardiac failure, subacute bacterial endocarditis	Spleen, liver, heart brain, parathyroid glands, stomach, colon, adrenal glands, kidneys
Dillon and Evans 13	1942	52	M	Peripheral edema, pains and paralysis of legs	Chronic nephritis with edema (nephroses), phlebitis of right leg	1 yr	Terminal pneumonia	Kidneys, spleen, liver, adrenal glands
Lindsay and Knorp	1945	60	M	Pain in thoracic part of spine, loss of weight, nasal obstruction and hemorrhage, epigastric and substernal pain, orthopnea and dyspnea	Gastric ulcer, coronary atherosclerosis with cardiac insufficiency	20 mo	Acute cardiac failure, myocardial infarction	Skin of ears, fingers and toes, endocardium and cardiac valves, pulmonary artery, trachea, mucosa, aorta, lymph nodes, bone marrow, nasal mucosa, adrenal glands, liver, spleen, pancreas, arteries and arterioles of pancreas, spleen, kidney, testes and stomach

after eating. These symptoms had been noted for approximately one year. He had been unable to continue his occupation as a carpenter because of the pain in his back. His family and past history were not significant, though he stated he had never felt well since the infection of the hand five years before.

The patient was a 60 year old man, weighing 134 pounds (60.5 Kg). His temperature was 98.4 F, his pulse rate, 74. A perforation of the anterior portion of the nasal cartilage was surrounded by abundant, soft pale, pink polypoid tissue, which was producing bilateral nasal obstruction. The mouth, the tongue, the pharynx and the larynx were normal. The jaws were edentulous. On the superior surface of the helix of each ear was an irregularly outlined moist red eczematous lesion, 1 cm in diameter. The nasal and aural lesions were submitted to biopsy and will be described on later pages. The external lymph nodes and the thyroid gland were not enlarged. There was marked thoracic kyphosis centering between the third and fifth dorsal vertebrae. The anterior-posterior and transverse diameters of the chest were equal.

The diaphragms moved well. The lung fields were clear. The heart was normal in size, the rhythm was regular, and the sounds were of good quality. No murmurs were heard. The blood pressure was 130 systolic and 80 diastolic. The abdomen, the rectum and the genitalia revealed nothing significant. Narrow dark pink longitudinal striations were seen beneath the nails of the fingers and the toes. The urine was normal. The Kline test was negative. Analysis of the gastric content gave values within normal limits.

Roentgen examination showed a large ulcer on the upper lesser curvature of the stomach. By fluoroscopy no peristaltic waves were noted in the gastric wall in the region of the ulcer. There was moderate diffuse osteoporosis of the thorax and the pelvis with accentuation of the trabecular markings. The thoracic vertebral bodies were narrowed anteriorly, leading to marked dorsal kyphosis. There was considerable thickening of the anterior intervertebral margins of these bodies.

After three weeks on a dietary regimen the patient was greatly relieved subjectively. The gastric ulcer was one quarter of the former size in subsequent roentgenograms.

At this time, however, the patient first complained of oppressive substernal pain with radiation down both arms. The occurrence of numerous similar attacks in the past was brought out by further questioning. The pain occurred during the day and was associated with considerable orthopnea and dyspnea. An electrocardiogram on April 1, 1942 showed a rate of 60 per minute, regular rhythm, upright P waves, a normal PR interval, slightly slurred QRS, slightly high ST segments in I and II, essentially flat ones in III and IV and upright T waves. This was interpreted as minimal evidence of myocardial damage.

On April 13 he entered the hospital complaining of pain in the left side of the chest, increased with respiration and associated with an elevated temperature, malaise, cough and hemoptysis of a week's duration. Examination showed an ill elderly man, coughing at intervals and expectorating blood-streaked sputum. His temperature was 102 F. The nasal mucosa showed the same changes that were noted previously. There was redness of the pharynx. The lymph nodes were not enlarged. Dulness and diminished breath sounds were present over the lower lobe of the left lung. Coarse rhonchi were heard posteriorly over the lower lobe of the right lung. The heart was centrally placed

and normal in size, the rhythm was regular, and the rate was 95.

A roentgenogram showed an area of infiltration in the lower half of the lower lobe of the left lung. The bronchovesicular markings were slightly accentuated.

The urine contained the faintest possible trace of albumin. The white blood cell count was 25,800, with polymorphonuclears 47 per cent (12 per cent nonsegmented), lymphocytes 50 per cent and monocytes 3 per cent. A diagnosis of acute bronchitis with bronchopneumonia and atelectasis of the lower lobe of the left lung was made. Sulfathiazole was administered, 1 Gm every four hours for four days and 1 Gm every eight hours for four days. His temperature returned to normal on the third day after entry, and except for a rise to 100 F on the sixth day, remained normal thereafter. During his hospital stay he had frequent attacks of intense boring substernal pain, not aggravated by movement but relieved by glyceryl trinitrate. On several occasions the urine showed a faint trace of albumin and numerous sulfathiazole crystals. The white blood cell count gradually decreased to 8,500, with polymorphonuclears 31 per cent (2 per cent nonsegmented), lymphocytes 65 per cent and monocytes 4 per cent. His red blood cell count remained low (3,200,000), with hemoglobin 78 per cent. He remained in the hospital for twenty-eight days.

He again entered the clinic, May 17, 1942, with a history of nausea, vomiting and diarrhea of three days duration. He had lost weight and was obviously ill. There were no other significant physical findings. The hemoglobin at this time was 60 per cent, but there was no blood in his stools. Rest in bed and continuation of the dietary regimen were advised.

By June 2 he had been relieved of his abdominal distress, though he still had an occasional episode of radiating pain in the chest. These attacks occurred particularly at night but also followed exertion during the day. The general condition appeared to be improved, the weight had increased, the heart and the lungs were normal and the blood pressure was 140 systolic and 85 diastolic.

On July 8 he stated that his gastric distress had recurred but that the episodes of thoracic pain had diminished in severity.

On August 10 marked malaise and dyspnea developed. The skin had a yellowish tinge, and enlarged bilateral cervical lymph nodes were palpable. Moist rales were heard in both lungs. The apical beat was felt in the left fifth intercostal space. A systolic murmur was heard over the entire precordium but was heard best at the apex. An occasional ectopic beat was noted. The right upper quadrant of the abdomen was tender. The edge of the liver was felt 6 to 7 cm below the right costal margin. The spleen was enlarged to 1 cm below the left costal margin. Because his condition did not improve at home, he was advised to enter the hospital.

On his last entry, August 15, his condition was unchanged. His pulse was thready and irregular, with a rate of 120. His respiratory rate was between 32 and 36. He was expectorating sanguineous yellowish sputum. The tongue was dry and coated. Fine moist rales were heard in both lungs. The apical beat was felt in the left fifth intercostal space, outside the left sternoclavicular line. A systolic murmur was heard at the apex over the aortic area. His temperature was 101 F on entry and remained above 100 F during his stay in the hospital. There was no response to digitalis, nifedipine or digifolin or to sedatives. Death occurred thirty-six hours after entry.

*Autopsy* (five hours after death)—The lesions of the ears and the nails of the fingers and the toes appeared as before death. External examination revealed no other significant abnormality.

The heart weighed 550 Gm. Its left border extended 11 cm to the left of the midline and its right border 5 cm to the right of the midline. The anterior surface of the left ventricle had a dark, congested, mottled appearance though the epicardium was intact. All the chambers of the heart were dilated. The left ventricle had a globular outline. The left ventricular wall averaged 1 cm in thickness, and its anterior portion showed a diffuse congested mottling with small yellowish opaque zones of early necrosis. Elsewhere the myocardium was normal. The right ventricular wall averaged 0.5 cm in thickness. The valves measured as follows: aortic, 8 cm; mitral, 11 cm; tricuspid, 12 cm; pulmonary, 9 cm.

All the valves and much of the endocardium in all the chambers of the heart showed a remarkable alteration (figs 1 and 2). Covering a large part of the valve surfaces and extending onto the chordae tendineae and adjacent endocardial surfaces were elevated confluent nodular masses of smooth glistening, translucent yellowish white, soft amyloid material. On the left side of the heart this material was most abundant on the under surface of the aortic leaflet of the mitral valve and extended upward beneath and onto the aortic cusps (fig 1). On the latter most of the deposition was along the free edges and in the commissures. In addition, the aortic cusps were thickened, fibrous and moderately rigid and contained yellow atheromatous deposits in their deeper portions. Smaller nodules and deposits of similar translucent material were present throughout the left ventricular chamber, projecting from the endocardium. Both small and large endocardial plaques were present in the left auricle. On the right side of the heart the valvular alteration was even more extensive. Both on the tricuspid and on the pulmonary valve (fig 2) the translucent deposits were more elevated and more nodular and covered more of the valvular surfaces. Deposits on the chordae tendineae had marked nodularity. In these situations the fibrous structure of the chordae tendineae was visible through the overlying translucent amyloid layer. This material extended distally from both the aortic and the pulmonary valve to involve the intima of the aorta and that of the pulmonary artery (fig 3). The involvement of the latter was greater. There were small nodular amyloid deposits in the endocardium of the right auricle and ventricle. There was marked atherosclerosis of the right and left coronary arteries with narrowing of the lumen of each vessel. Complete obstruction could not be demonstrated.

Atherosclerosis of the aorta with ulceration and calcification was most marked in the arch and in the abdominal portion. In addition there was extensive intimal deposition of glistening pale gelatinous material in all portions of the aorta. The vena cava was normal.

The pericardial cavity was normal. There was 1,500 cc of clear yellow fluid in the right pleural cavity, 500 cc of similar fluid in the left pleural cavity and 1,500 cc in the abdominal cavity.

The larynx, the thyroid gland, the trachea and the bronchi were not altered. The extensive confluent nodular intimal deposits in the pulmonary artery extended well down into the smaller branches of the pulmonary artery. Both lungs were congested and moderately edematous. In each lower lobe were sev-

eral small triangular-shaped dark red firm subpleural infarcts.

The liver weighed 2,200 Gm and extended 4 cm below the xiphoid process and the right costal margin. The anterior edges were rounded. The parenchyma was pale with marked central congestion. The gall bladder wall was edematous. The spleen was enlarged and weighed 680 Gm. The pulp was soft, hyperplastic and light purple.

The adrenal glands, the kidneys, the renal pelves, the ureters, the bladder, the prostate gland and the testes were normal.

The gastroenteric tract was normal except for the stomach. On the lesser curvature, 4 cm from the cardioesophageal junction, was a large ulcer measuring 4 by 5 cm in diameter (fig 4). Its edges were elevated, sloping and indurated. Abundant glistening yellowish white soft gelatinous amyloid material lined the crater. Perforation had occurred, extending through almost the entire wall. The serosal surface was scarred and contracted.

A group of enlarged amyloid-containing lymph nodes forming a mass measuring 12 cm in diameter lay just above the head of the pancreas (fig 5). Strong solution of iodine U. S. P. stained the amyloid material in these nodes light brown. Similar but smaller nodes lay along the lesser curvature of the stomach and along the abdominal portion of the aorta.

The mesentery of the small bowel was thickened and its surface mottled and congested. Tending to surround the mesenteric lymph nodes were rounded zones of opaque, yellowish white fat necrosis. The larger mesenteric arteries had small intimal deposits of pale, translucent amyloid material.

The marrow space of the medial portion of the clavicle consisted entirely of amyloid material.

The spine and the cranial cavity were not examined.

After several days' fixation in a solution of formaldehyde the amyloid material in all the tissues underwent considerable shrinkage and took on a brownish purple color but retained its glistening translucent characteristics.

*Microscopic Description*—The pericardial fat contained many small collections of lymphocytes, plasma cells and large mononuclear cells. There was considerable interstitial myocardial edema. Some groups of myocardial fibers had been replaced by a cellular fibrous tissue, while other groups within the left ventricle were necrotic and surrounded by neutrophilic leukocytes. The endothelium of the ventricles and valves was intact. The subendothelial layer was widened, contained an unusually rich network of reticulum fibers and consisted of eosinophilic amorphous homogeneous material in which there were scattered compressed fibrocytes. The connective tissue fibers demonstrated with the Mallory and Jaidlaw connective tissue stains, separated the amyloid into irregular rounded masses. There were no myocardial or pericardial amyloid deposits.

All the heart valves presented an identical histologic appearance (fig 7). Lying between the intact endothelium and the central fibrous portion of the valve was a wide layer of eosinophilic amorphous homogeneous amyloid material containing scattered compressed fibrocytes. As in the endocardium, this material was well supplied with reticulum and collagen fibers. There was no increased vascularity of the valves. A minimal lymphocytic infiltration was present in the deeper areas. The distal portions showed more extensive deposits than the proximal portions. The

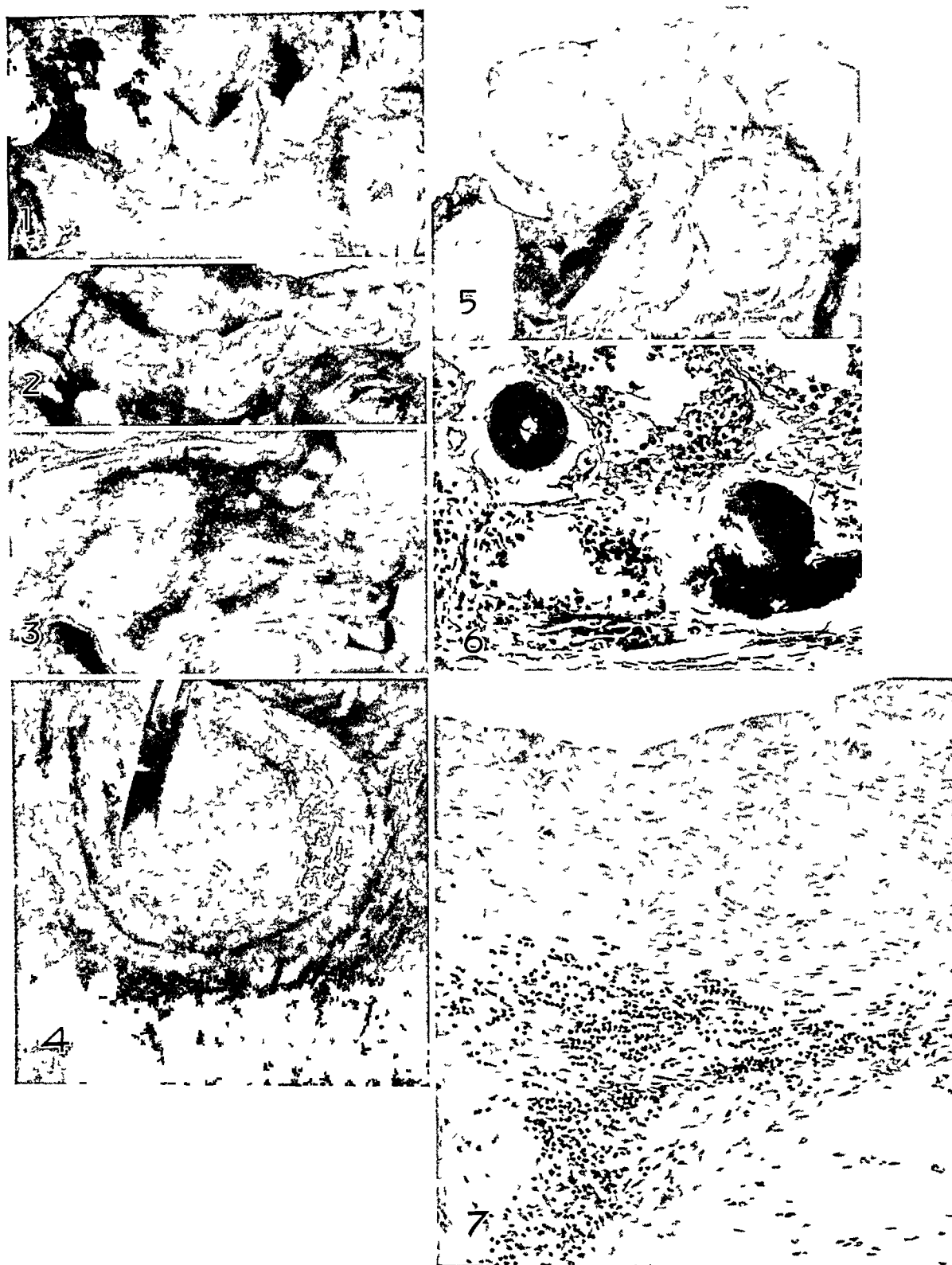


Fig 1—Left ventricle showing deposits of amyloid in the aortic and mitral valves,  $\times 06$

Fig 2—Pulmonary valve with extensive deposits of amyloid,  $\times 06$

Fig 3—Pulmonary artery showing extensive deposits of amyloid in the intima,  $\times 11$

Fig 4—Gastric ulcer with amyloid material visible in the crater of the ulcer,  $\times 1$

Fig 5—Peripancreatic lymph nodes with extensive deposits of amyloid  $\times 07$

Fig 6—Small testicular arteries with amyloid infiltration, hematoxylin and eosin stain,  $\times 120$

Fig 7—Subendothelial amyloid infiltration of a cardiac valve hematoxylin and eosin stain,  $\times 120$

small myocardial arteries were not altered. The large coronary arteries showed extensive intimal atherosclerosis with marked narrowing of the lumens. Contributing in a small degree to this narrowing were subendothelial deposits of amyloid material. There was no thrombosis.

The pulmonary artery and its branches showed between the endothelium and the media a wide zone of deposition of amyloid. This contained a few compressed fibrocytes and small collections of lymphocytes. The lumens of the involved smaller vessels were narrowed. The smallest pulmonary arteries were not altered. The alveolar walls were fibrous and congested. The alveolar spaces contained serous fluid, fibrin and pigment-containing macrophages. There was no inflammatory reaction and no deposition of amyloid. Zones of early hemorrhagic infarction were present in the lower lobes.

The hepatic sinusoids were wide but contained few red blood cells. The parenchymal cells were compressed, and many contained small lipid globules. A few groups of central cells were undergoing necrosis. Occasional small deposits of amyloid were present in portal spaces. There was marked edema of the gall-bladder wall.

The spleen contained numerous large malpighian follicles. The pulp was cellular and consisted almost entirely of lymphocytes and reticuloendothelial cells. Neutrophilic leukocytes were present in moderate numbers. Deposits of amyloid were seen in about one half of the central arterioles. They were most abundant beneath the endothelium and in the adventitia. Smaller deposits of amyloid were noted beneath the splenic capsule.

There was subendothelial amyloid infiltration in the large and the small pancreatic arteries. Beneath the amyloid layer were large atheromatous deposits. The pancreatic parenchyma was not altered except for an occasional small amyloid mass in the interstitial tissues with no apparent relation to blood vessels.

The renal parenchyma was not significantly altered except for small deposits of amyloid in a few afferent arterioles.

The bladder and the prostate gland were not altered.

In the testes were many altered blood vessels consisting of an inner layer of endothelium, a wide zone of amyloid material and a thin adventitial layer (fig. 6). A moderate degree of spermatogenesis was noted, and the interstitial cells were normal.

All the lymph nodes examined contained deposits of amyloid. These were particularly extensive in the peripancreatic group of nodes, where little lymphatic tissue remained. The amyloid material was present as rounded concentric masses separated by narrow clefts containing compressed fibrocytes and small lymphocytic collections. In nodes with less involvement the amyloid lay adjacent to the endothelium of blood vessels and sinusoids, particularly those of the subcapsular group.

The small and the large bowel were normal. The gastric mucosa was thin and atrophic. The few remaining gastric glands were irregularly arranged and were separated by many lymphocytic collections and masses of amyloid material. At the edge of the gastric ulcer the mucosa was thicker but still retained its disorganized structure. There was no epithelium in the crater of the ulcer. The gastric wall beneath was extensively infiltrated with amyloid material. This lay in the interstitial tissue, compressed the muscular layers, was deposited in the arterial walls and was accompanied by many lymphocytic collections.

Groups of fat cells in the mesentery of the small bowel were necrotic. These were surrounded by lipid-containing macrophages and lymphocytes.

Small interstitial deposits of amyloid were present in the adrenal cortices.

At various levels the aorta had essentially the same histologic appearance. The intima was irregularly widened by subendothelial amyloid infiltration. The atheromatous lesions present were between the amyloid liver and the media. There were a few lymphocytes around the vasa vasorum.

The acini of the thyroid gland were uniform in size. The central colloid stained more deeply than that at the periphery. In the interstitial tissue were small lymphocytic collections, small groups of lipid-containing macrophages and an occasional foreign body cell. No amyloid was encountered.

The trabeculae in the clavicular marrow were widely separated. The marrow spaces were almost entirely occupied by eosinophilic homogeneous amyloid material. Few myelocytic and erythrocytic elements were present. Megalokaryocytes and plasma cells were absent.

The biopsy specimens from the ear and the nose were reexamined after the patient's death. In both there was extensive deposition of amyloid in the sub-epithelial tissues.

At those sites where the amyloid material was abundant, it was deposited as rounded concentric masses separated by compressed fibrocytes, and contained a dense network of reticulum and collagen fibers. In the large arteries the cardiac valves and the endocardium a uniform relationship to the endothelium could be established. In the small vessels, primarily arterial the material left no residual mural tissue and led to marked narrowing of the lumens.

A few small arteries showed another type of alteration. These vessels contained no deposits of amyloid but showed distinct edema of the vessel wall with separation of the muscular and the connective tissue elements and exfoliation of the endothelium.

*Observations with Special Staining Methods*—Tissues prepared with congo red, crystal violet and Mayer's iodine stains gave variable results when compared with control tissues presenting secondary amyloidosis. In general, the amyloid material stained less deeply and distinctly than that in secondary amyloidosis with all these stains. Of these, the congo red stained the amyloid more deeply. There was considerable variation in staining intensity with the same stains in different tissues. The amyloid material appeared a brilliant red with the Mallory stain, though in the blood vessels the amyloid material at the periphery had an orange color. The Laidlaw connective tissue stain demonstrated a fine reticulum network in the amyloid deposits. This was not apparent with the routine hematoxylin and eosin stain.

*Pathologic Diagnosis*—Primary systemic amyloidosis, with involvement of the endocardium and the cardiac valves, the pulmonary artery and its branches, with multiple pulmonary infarcts, the aorta, the gastric mucosa, with atrophic gastritis and chronic gastric ulcer, the lymph nodes, the bone marrow, the arteries and arterioles of the pancreas, the spleen, the kidneys, the testes and the stomach, the adrenal glands, the liver, the spleen, the pancreas, the skin of the ears, and the nasal mucosa. Generalized arteriosclerosis with aortic and aortic valvular atherosclerosis and coronary atherosclerosis followed by myocardial infarction of the left ventricle with myocardial failure, producing

pulmonary congestion, bilateral hydrothorax, hepatic congestion and ascites Fat necrosis of the mesentery of the small bowel

## COMMENT

Reimann, Koucky, and Eklund<sup>14</sup> have proposed a simple clinicopathologic classification of amyloid disease: primary amyloidosis, secondary amyloidosis, tumor-forming amyloidosis and amyloidosis associated with multiple myeloma.

The primary form is characterized by (a) involvement of the mesodermal tissues, the cardiovascular system, the gastroenteric tract, the smooth and the striated muscle and the lymph nodes, (b) little or no involvement of those tissues usually containing deposits of amyloid in the secondary form, (c) formation of nodular masses of amyloid, (d) deposition of amyloid that shows atypical staining reactions and (e) absence of preceding infection.<sup>15</sup>

The secondary form is the most common; it follows chronic suppurative disease, is characterized by deposits of amyloid that give a typical reaction to amyloid stains and extensively involves the liver, the spleen, the kidneys and the adrenal glands.

Tumor-forming amyloidosis belongs to the primary type but is characterized by solitary or multiple amyloid tumors in the respiratory tract and other situations.<sup>16</sup>

Amyloidosis occurring with multiple myeloma is characterized by a distribution similar to that seen in the primary type, though large deposits may be found in the joints and elsewhere.<sup>17</sup>

As more cases of all types are recorded, it is apparent that the characteristics of each type merge considerably.<sup>18</sup> In addition the cases recorded by Budd<sup>19</sup> and Spain and Barrett<sup>20</sup> presented a mesodermal distribution of amyloid characteristic of the primary type though the disease occurred secondary to chronic suppuration. In other cases in which there was a mesodermal distribution, the supposed primary inflammatory process may have been coincidental.<sup>21</sup>

<sup>14</sup> Reimann, H. A., Koucky, R. F., and Eklund, C. M. *Am J Path* **11** 977, 1935.

<sup>15</sup> Strauss, A. *Virchows Arch f path Anat* **291** 219, 1933, cited by Reimann, Koucky and Eklund.<sup>14</sup> Lubarsch.<sup>1</sup>

<sup>16</sup> Von Bonsdorff, B. *Finska lak-salsk handl* **75** 447, 1933, abstracted, *J A M A* **101** 489, 1933, cited by Reimann, Koucky and Eklund.<sup>14</sup>

<sup>17</sup> Magnus-Levy, A. *Ztschr f klin Med* **126** 62, 1933, cited by Reimann, Koucky and Eklund.<sup>14</sup>

<sup>18</sup> Koletsky and Stecher.<sup>2</sup> Reimann and others.<sup>14</sup>

<sup>19</sup> Budd, J. W. *Am J Path* **10** 299, 1934.

<sup>20</sup> Spain, D. M., and Barrett, R. C. *Arch Path* **38** 203, 1944.

<sup>21</sup> Königstein, H. *Arch f Dermat u Syph* **148** 330, 1925, cited by Koletsky and Stecher.<sup>2</sup> Israel.<sup>7</sup>

The separation of primary isolated amyloidosis and primary systemic amyloidosis seems artificial. While the bulk of the amyloid material may be present in one or two organs, deposits are almost invariably found in other tissues.

The origin of amyloid material in both the primary and the secondary form of amyloidosis remains obscure. Hass and Schulz<sup>22</sup> and Hass<sup>23</sup> have shown that amyloid is composed of two protein fractions and one polysaccharide fraction. It has been suggested that the deposition of amyloid may be the result of a reaction between a component of the serum globulin and fixed tissue elements in the vascular walls, i. e., an antigen-antibody reaction.<sup>2</sup> Amyloid material then may be a precipitate resulting from this union. Presumably this reaction occurs in or on vascular lining cells.<sup>24</sup> Moreover, the relation of amyloid to vascular endothelium is particularly striking both in the primary<sup>5</sup> and in the secondary form of the disease. Because of the overlapping of the four general types of amyloid disease, it is possible that in the future hypersensitivity states other than bacterial may be implicated in the so-called primary type. Sensitivity to drugs or foods may be responsible.

The valvular and endocardial deposits in this case were extensive, particularly on the right side of the heart. The resulting thickening and rigidity undoubtedly contributed in part at least to the cardiac failure. While there were small endothelial deposits of amyloid in the main coronary arteries, the narrowing of these vessels and the subsequent myocardial infarction were the result of atherosclerosis. Although the heart has been the site of deposition of amyloid in most of the cases reported,<sup>2</sup> valvular involvement is usually slight. In the cases described by Wild,<sup>25</sup> Koletsky and Stecher,<sup>2</sup> Silver and Lindblom,<sup>4</sup> Israel,<sup>7</sup> and Koller,<sup>6</sup> however, considerable deposition of amyloid had occurred in the cardiac valves.

The amyloid present in the aorta and the pulmonary artery had the same subendothelial distribution as that in the heart. Where aortic atheromatous lesions were encountered these lay beneath the amyloid deposits. In Larsen's<sup>5</sup> case the aortic amyloid had a medial distribution, while in the second case described by Pearson, Rice and Dickens<sup>11</sup> the adventitia was the site of maximum deposition.

<sup>22</sup> Hass, G., and Schulz, B. Z. *Arch Path* **30** 240, 1940.

<sup>23</sup> Hass, G. *Arch Path* **34** 92, 1942.

<sup>24</sup> Cannon, P. R., Walsh, T. E., and Marshall, C. E. *Am J Path* **16** 682, 1940.

<sup>25</sup> Wild, C. *Beitr z path Anat u z allg Path.* **1** 177, 1886, cited by Koletsky and Stecher.<sup>2</sup>

One of the patient's complaints was severe pain of the back. Narrowing of the thoracic vertebral bodies with resulting kyphosis was probably due to deposition of amyloid in the vertebral marrow.<sup>6</sup> The clavicular marrow was almost entirely replaced by amyloid material. While other portions of the skeleton were not examined, more widespread involvement was likely. The anemia and persistent mild relative leukopenia may have been related to replacement of marrow.

Involvement of the skin was shown in 8 of the 24 cases collected by Koletsky and Stecher.<sup>2</sup> The skin was involved in a case in Pearson's<sup>11</sup> series, and in the cases described by Israel<sup>7</sup> and Sappington and co-workers.<sup>12</sup> The cutaneous lesions are usually nodular or papular and frequently are waxy and translucent. At times they resemble scleroderma. In this case the lesions in the skin were confined to the upper surfaces of the external ears. Each was of the weeping eczematous type. The amyloid was not recognized in the biopsy prior to the autopsy, though the bizarre history and the objective findings suggested that the aural lesion may have been one manifestation of a diffuse process. The nasal amyloid had an identical subepithelial distribution. The longitudinal striations beneath the nails of the fingers and the toes were not examined histologically but undoubtedly were amyloid in nature.

Amyloid involvement of the oral mucosa, the tongue, the pharynx and the larynx has been described frequently.<sup>13</sup> In this case the amyloid

infiltration of the nasal mucosa led to hemorrhage, septal perforation and bilateral nasal obstruction.

The gastroenteric involvement was limited to the stomach. There was resulting mucosal atrophy of the entire organ. The extensive deposition of amyloid in and adjacent to the gastric ulcer indicates an amyloid basis for the ulceration. In a case in Lubarsch's<sup>1</sup> series amyloid disease of the stomach was accompanied by a number of pyloric ulcers presumably due to deposition of amyloid.

Abundant amyloid infiltration within the peripancreatic group of lymph nodes produced an amyloid tumor. This again demonstrated the merging of the characteristics of the four types of amyloid disease.

#### SUMMARY

Forty cases of primary systemic amyloidosis have now been recorded in the literature.

In the case of primary systemic amyloidosis recorded in this paper the illness was present for twenty months. There was extensive involvement of the endocardium and of all the cardiac valves, most marked on the tricuspid and pulmonary valves. Abundant amyloid material was also deposited in the skin, the nasal mucosa, the pulmonary arteries, the aorta, the lymph nodes, the stomach and the marrow. Less marked infiltration was present in the adrenal glands, the liver, the spleen, the pancreas and the arteries and arterioles of the stomach, the pancreas, the spleen, the kidneys and the testes. Death resulted from myocardial infarction secondary to coronary atherosclerosis.

<sup>26</sup> Mandl, J. *Virchows Arch f path Anat* **253** 639, 1924, cited by Koletsky and Stecher - Gerber.<sup>8</sup>



# PROLIFERATION OF MUSCLE CELLS IN THE MYOMETRIUM OF THE NONPREGNANT UTERUS

PHILIP H HARTZ, MD  
Pathologist of the Public Health Service  
CURAÇAO, NETHERLANDS WEST INDIES

The question whether completely differentiated unstriated muscle cells retain the ability to divide by mitosis is still being discussed. Maximow and Bloom,<sup>1</sup> though admitting that mitosis may occur, expressed the belief that the capacity for regeneration is small. Stieve<sup>2</sup> never observed mitotic division in the muscle cells of the pregnant uterus, and Meyer<sup>3</sup> stated that it is "a generally accepted fact that mitoses do not occur in fully developed muscle cells with myofibrils."

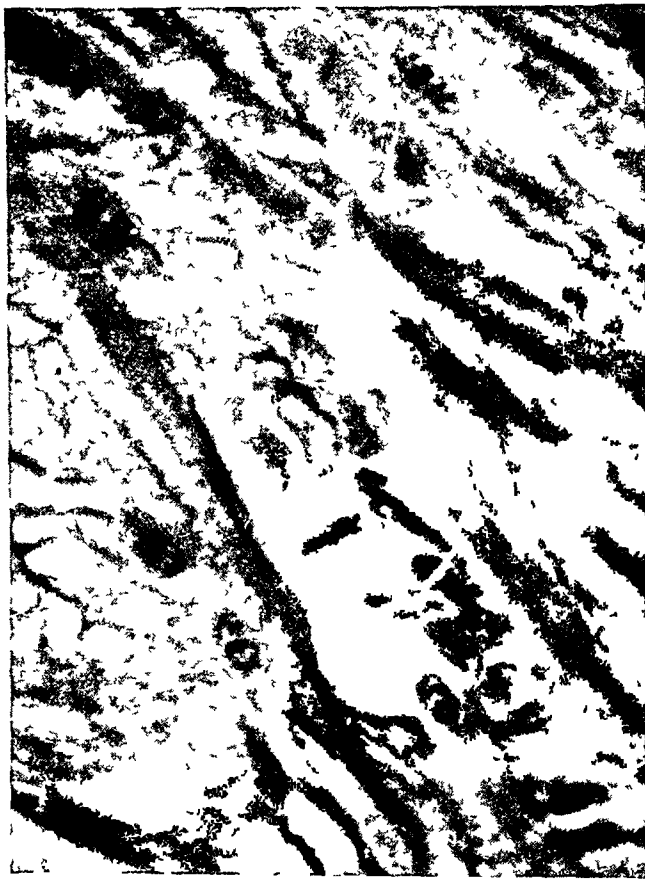
In an article on the mitotic activity in uterine leiomyoma Hartz and Hugenholtz<sup>4</sup> demonstrated that mitosis is common in the muscle cells composing tumors of that type and occurs in fully differentiated cells with distinct myofibrils. As the stigma of tumor cells remains attached to the cells of myoma and as they cannot be considered as absolutely normal muscle cells, it is logical to extend the search for mitosis of muscle cells to uteri with hypertrophy of the myometrium, with use of the histologic methods suitable for the preservation of the finer details of the muscle cells and the mitotic figures.<sup>4</sup> That such proliferation of the muscle cells in myometrial hypertrophy occurs is shown in the case which I shall now describe.

## REPORT OF A CASE

A Negro woman, 41 years old, gave the following history. One day before the expected date of menstruation (April 21, 1944) the flow had started and had been much stronger than usual. The menstruation had always been regular and normal. She had had two normal deliveries, the last one sixteen years before, after the birth of the second child, but many years ago, she had had a miscarriage. Appendectomy had been performed one year previously. On the clinical

diagnosis of myoma uteri supravaginal hysterectomy was performed on May 5, 1944 (Dr F R van de Stadt). The ovaries and tubes showed nothing abnormal. The postoperative course was uneventful. The uterus was enlarged, the anterior wall contained an almost completely necrotic myoma, about 6 cm in diameter. The myometrium was thickened and pale, rose-white, the consistence was diminished. The endometrium was thin and showed nothing abnormal.

*Microscopic Examination*—(a) Myoma. The myoma was almost completely necrotic, it contained much con-



A muscle cell during mitosis. The centrally located myofibrils can be easily distinguished from the paler peripheral protoplasm. Masson's tetrachrome,  $\times 600$ .

1 Maximow, A. A., and Bloom, W. A Textbook of Histology, ed 4, Philadelphia W B Saunders Company, 1942, p 161.

2 Stieve, H., cited by Meyer<sup>3</sup>.

3 Meyer, R. Uterus und Tuben, in Henke, F., and Lubarsch, O. Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1930, vol 7, pt 2, p 632.

4 Hartz, P. H., and Hugenholtz, M. J. Am J Clin Path 12 523, 1942.

nective tissue. Only in the peripheral parts could a few viable muscle cells be found, they were much thinner than the muscle cells of the myometrium.

(b) Endometrium. The endometrium was relatively thin, and the cells of its stroma were loosely arranged. The glandular epithelium showed no signs of secretion. There were a few lymphocytes in the stroma. The veins were wide. The endometrium was evidently in the proliferative phase, as mitotic divisions occurred in

the cells of the stroma, in the surface epithelium and in the epithelium of the glands. There was slight endometriosis interna.

(c) Myometrium. The myometrium was composed of the usual bundles of unstriated muscle cells, separated by loose connective tissue carrying the blood vessels and containing a few fibrocytes and histiocytes and an occasional lymphocyte or leukocyte. The muscle cells were large and broad and resembled the muscle cells of the pregnant uterus. All of them contained many distinct myofibrils, which were beautifully brought out by the Masson stain. Mitotic division occurred throughout the preparations, although its frequency varied from one place to another. Sometimes two or three mitotic figures could be counted in one field of a 2 mm objective, in other places they were less frequent, especially in the stratum submucosum. The cells in mitosis showed all the characteristics of the mitotic muscle cells which we have already described,<sup>4</sup> especially the rounded swelling in the nuclear area and the interruption of the myofibrils by the perinuclear protoplasm. Another peculiarity, which was especially visible in the broad cells, was the tendency of the fibrils to group themselves centrally in the cell during mitosis, thus leaving the peripheral protoplasm free. I have also observed this phenomenon in mitosis of muscle cells in the media of small arteries and in myomas, according to my experience it is typical for the unstriated muscle cell. The myofibrils could be demonstrated in all dividing cells. The other special structures which belong to, or are particularly visible during, mitosis, such as achromatic spindles, centrosomes and, in the telophase, Flemming's intermediate body, were distinct. The chromosomes were perhaps a little larger than in the mitotic muscle cell in myoma. In the connective tissue no signs of proliferative activity could be observed, apart from an isolated mitotic figure in the endothelium of a small artery.

#### COMMENT

As follows from the foregoing description, mitotic figures occurred in a myometrium which on gross examination appeared hypertrophic. That pregnancy was not the cause of the proliferation of the muscle cells was proved by the gross and the microscopic examination of the endometrium. Likewise, good care was taken not to confound myometrium with myoma, and the possibility that the observation in reality concerned a very small, grossly invisible myoma with cells in mitosis is excluded by the fact that

the mitotic figures occurred in a large area of the myometrium. That the myoma was the cause of the myometrial hypertrophy is not probable, as such hypertrophy is not common in the large myoma material of the Public Health Service laboratory in Curaçao, especially not in women of this age. The necrosis of the myoma and the hypertrophy are perhaps caused by the same factor, as happens in pregnancy. Hypertrophy of the myometrium such as that observed in this case is a regular finding in the clinical syndrome often named fibrosis uteri or metrorrhagia myopathica, which has recently been described by Williams and Kinney<sup>5</sup> as myometrial hypertrophy. In 10 cases in which specimens were examined microscopically these authors did not find muscle cells in mitosis, and they expressed the belief that the increase in size of the uterus is due to increased size of the muscle fibers of the myometrium and that the condition may represent an estrogenic effect. From a theoretic point of view it is important that the dividing muscle cells in this case showed many distinct myofibrils and did not differ in any respect from the rest of the muscle cells. They cannot, therefore, be classified as immature cells and thus there was marked proliferation of completely developed muscle cells without the presence of so-called myoblasts and partially developed muscle cells or intermediate forms between connective tissue cells and muscle cells. It must also be considered probable that proliferation of muscle cells plays a more important role in other forms of hypertrophy of the myometrium than is generally assumed.

#### SUMMARY

In the uterus of a 41 year old woman myometrial hypertrophy and a necrotic myoma were found. Microscopically, the myometrium showed numerous fully developed muscle cells, containing distinct myofibrils, in mitosis. Some characteristics of the mitosis of muscle cells are described.

<sup>5</sup> Williams, J. T., and Kinney, T. D. *Am J Obst & Gynec* 47: 380, 1944.

# PERIARTERITIS NODOSA-LIKE LESIONS IN RATS FED THIOURACIL

DAVID MARINE, M.D., AND EMIL J. BAUMANN, PH.D.  
NEW YORK

The occurrence of periarteritis nodosa-like lesions in animals has been reported by many observers—in a herd of axis deer by Lupke<sup>1</sup> and Jaeger<sup>2</sup>, in cattle by Nieberle,<sup>3</sup> Trawinski,<sup>4</sup> Knosel<sup>5</sup> and Guldner<sup>6</sup>, in swine by Joest and Harzer<sup>7</sup> and Nieberle<sup>8</sup>, in dogs by Baló,<sup>9</sup> and in rats by Wilens and Sproul.<sup>10</sup> The last-named observers found typical periarteritic lesions in 97 per cent of 487 rats dead of natural causes. All the involved rats were old ones—the females averaging 856 and the males 700 days of age, 30, or 63.8 per cent, were females.

Following the original account of the disease in man by Kussmaul and Maier,<sup>11</sup> there was a general tendency to consider syphilis as a cause. Then for a period it was considered as a specific infection, probably a virus infection, and Haun<sup>12</sup> and Harris and Friedrichs<sup>13</sup> reported the production of lesions resembling periarteritis nodosa in guinea pigs and rabbits, respectively, by inoculating them with blood or with saline extracts of organs from persons who had the disease. These observations have not been confirmed.

Following the studies of Gruber,<sup>14</sup> who came to the conclusion that the disease was a mani-

festation of hypersensitivity, all later observers have accepted his general conclusion that the vascular reaction is not a response to a single excitant but rather the expression of hypersensitivity in the vessel wall where a preceding infection or intoxication has rendered the wall locally hypersensitive. This view is also supported by the finding of vascular lesions similar to periarteritis nodosa in fatal cases of serum sickness.

Recently Rich<sup>15</sup> and Rich and Gregory<sup>16</sup> have reviewed the subject and brought further evidence, both from the spontaneous occurrence of the disease in human beings and from the experimental production of similar lesions in rabbits with injections of foreign serums, that the disease is always an allergic reaction. Rich has also emphasized the possible association of hypersensitivity to the sulfonamide drugs with the disease. He reported a case of periarteritis nodosa following the use of sulfathiazole alone. The patient received the drug for five days when it was discontinued, and a second course of treatment was started with the same drug after an interval of seven days. Following this, the patient's condition grew worse rapidly, and he died on the nineteenth day after the first dose of sulfathiazole. This patient also had widespread focal inflammatory necrotic lesions in the viscera of the type described by Lederer and Rosenblatt.<sup>17</sup>

## OBSERVATIONS ON LESIONS IN RATS FED THIOURACIL

Our special interest in this subject was the incidental finding of typical periarteritis nodosa-like lesions in 3 white rats, during the course of experiments on the effects of prolonged feeding with thiouracil (synthesized from thiourea). The principal data follow.

**RAT 100**—A young male adult, born in the laboratory and never used for other experiments, was fed 8 Gm

From the Laboratory Division, Montefiore Hospital. Thiouracil was generously supplied by Lederle Laboratories.

1 Lupke, F. Verhandl. d. deutsch. path. Gesellsch. 10: 149, 1906.

2 Jaeger, A. Virchows Arch. f. path. Anat. 197: 71, 1909.

3 Nieberle, K. Virchows Arch. f. path. Anat. 269: 587, 1928.

4 Trawinski, A. Arch. f. wissenschaftl. u. prakt. Tierh. 59: 207, 1929.

5 Knosel, W. Ztschr. f. Fleisch- u. Milchhyg. 41: 413, 1931.

6 Guldner, E. Virchows Arch. f. path. Anat. 219: 366, 1915.

7 Joest, E., and Harzer, J. Beitr. z. path. Anat. u. z. allg. Path. 69: 85, 1921.

8 Nieberle, K. Arch. f. wissenschaftl. u. prakt. Tierh. 76: 47, 1940.

9 Baló, J. Virchows Arch. f. path. Anat. 248: 337, 1924.

10 Wilens, S. L., and Sproul, E. E. Am. J. Path. 14: 201, 1938.

11 Kussmaul, A., and Maier, R. Deutsches Arch. f. klin. Med. 1: 484, 1866.

12 Haun, F. Virchows Arch. f. path. Anat. 227: 90, 1920.

13 Harris, W. H., and Friedrichs, A. V. J. Exper. Med. 36: 219, 1922.

14 Gruber, G. B. Virchows Arch. f. path. Anat. 258: 441, 1925.

15 Rich, A. R. Bull. Johns Hopkins Hosp. 71: 123 and 375, 1942.

16 Rich, A. R., and Gregory, J. E. Bull. Johns Hopkins Hosp. 72: 65, 1943.

17 Lederer, M., and Rosenblatt, P. J. A. M. A. 118: 8, 1942.

daily of our regular diet containing 40 mg (0.5 per cent) of thiouracil beginning on Nov 16, 1943. This diet consists of hominy, rolled oats, meat scrap, dried milk, dried brewers' yeast and salt moistened with milk. In addition fresh greens—usually lettuce—were given daily. This rat was kept in a wire bottom cage with 5 other males. They were transferred to a clean sterilized wire bottom cage each week. This animal remained fairly

day of thiouracil feeding a blood count showed 17,700 leukocytes and 5,200,000 erythrocytes. The rat was killed with chloroform and an autopsy made at once.

The body weight was 262 Gm. The thyroid gland weighed 0.172 Gm, it was dark red and slightly granular. The thymus was involuted, and there were several enlarged lymph nodes in the area. The heart weighed 1.02 Gm. The middle lobe of the right lung



Fig 1 (rat 100) —A segment of the mesentery. Note the aneurysmal dilatations and the thick-walled, congested and thrombotic arteries.

Fig 2 (rat 100) —A medium-sized mesenteric artery with intact media and intima, showing a branch artery cut lengthwise. The latter shows fibinoid necrosis of the subintima and the media, ending abruptly at its orifice. The endothelial lining is intact. There are moderate infiltration and fibrosis of the adventitia, more marked in the branch artery.

Fig 3 (rat 100) —A medium-sized artery in the pancreas showing advanced lesions with a complete ring of fibinoid necrosis and extensive cellular infiltration and fibrosis of the media and the adventitia. Adjacent is a portion of the wall of a large artery without involvement.

active, ate well and had a clean coat until about Nov 15, 1944, when scales were noted and its fur was erect. On December 6, the three hundred and eighty-sixth

was completely, and the lower lobe partially, consolidated. The upper lobe of the left lung showed patches of recent consolidation and was reddish, while the lower

lobe was completely consolidated and greenish gray, similar to the middle lobe. The spleen weighed 0.44 Gm. The liver was grayish purple. The pancreas was firm and edematous. The adrenal glands weighed 0.035 Gm and were grayish brown. The kidneys weighed 2.02 Gm. Their cortices were slightly pitted. No calculi were found in the urinary tract. The testes weighed 0.78 Gm, they were small but had normal outlines. The prostate and seminal vesicles together weighed 0.39 Gm. The hypophysis weighed 0.008 Gm. The mesentery (fig 1) from the duodenum to the cecum was thickened and

nodes, but the inguinal nodes were not enlarged. The aorta appeared normal.

*Microscopic Examination*—The changes in organs which we considered definitely associated with thiouracil feeding were marked hyperplasia of the thyroid gland, involution of the adrenal cortex, hypertrophy of the adrenal medulla, shrinkage of the spleen, the testes, the prostate and the seminal vesicles and parenchymatous changes in the liver and the kidneys. The pneumonia was of the chronic snuffles type with a recent acute hemorrhagic bronchopneumonic spread. Enlargement of

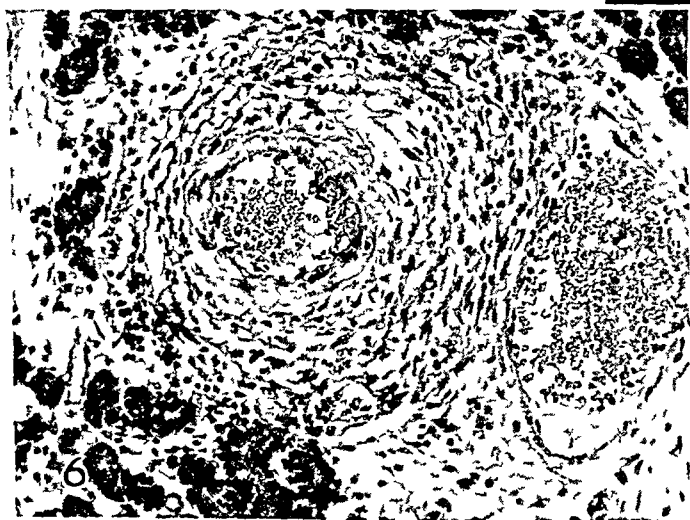
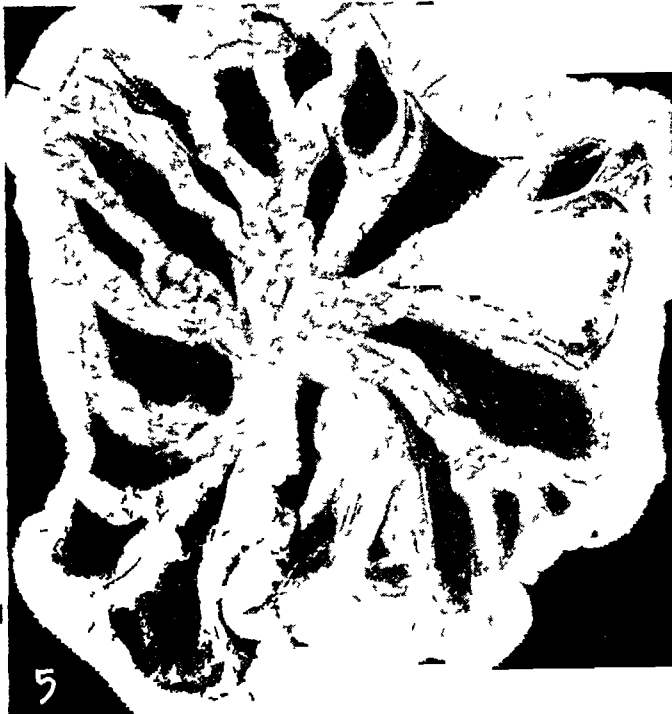


Fig 4 (rat 151)—A segment of the mesentery. There is only slight nodular thickening, masked by the normal fat.

Fig 5 (rat 34a)—A segment of the mesentery. Note the thickened nodular arterial cords.

Fig 6 (rat 151)—A cross section of a small artery in the pancreas with very early fibrinoid necrosis visible at one point with characteristic thickening and cellular infiltration of the adventitia. The accompanying vein is normal.

Fig 7 (rat 151)—A cross section of a small artery in the pancreas with very early perivascular infiltration. The accompanying vein is normal.

indurated, with no visible fat and tortuous nodular cords fanned out from the base to the intestinal wall. These nodules were grayish brown and on section contained both fluid and clotted blood. The branches of the mesenteric vein appeared normal. There was slight enlargement of the mesenteric and peripancreatic lymph

the mediastinal lymph nodes is always present in pneumonia. Enlargement and hyperemia of mesenteric lymph nodes, however, are usually not present in pneumonia and should, we believe, be considered as part of the periarteritis complex. The periaarterial lesions were limited to the arteries of the mesentery, the pancreas and

the retroperitoneal tissue at the base of the mesentery. The most advanced lesions were seen in the mesentery and the pancreas, where in any given section most of the arteries were involved, although one observed in any section a few relatively normal arterial walls. Figure 2 illustrates the local character of the lesion, which in this instance was limited to the branch artery at this level. The lesions were practically identical with those found in human periarteritis nodosa and need not be described in detail. In this rat there were

when the subintimal necrosis was extensive. However, it was over these areas of necrosis that one noted thrombus formation—sometimes fresh and at other times completely organized. The periarterial lesions were also present in all stages from acute edema and cellular infiltration to healing with extensive formation of granulation tissue. In this rat the edema, the cellular exudate and the formation of granulation tissue involved all of the mesentery and gave it the firm, thickened, semirigid condition noted in the gross examination. The walls

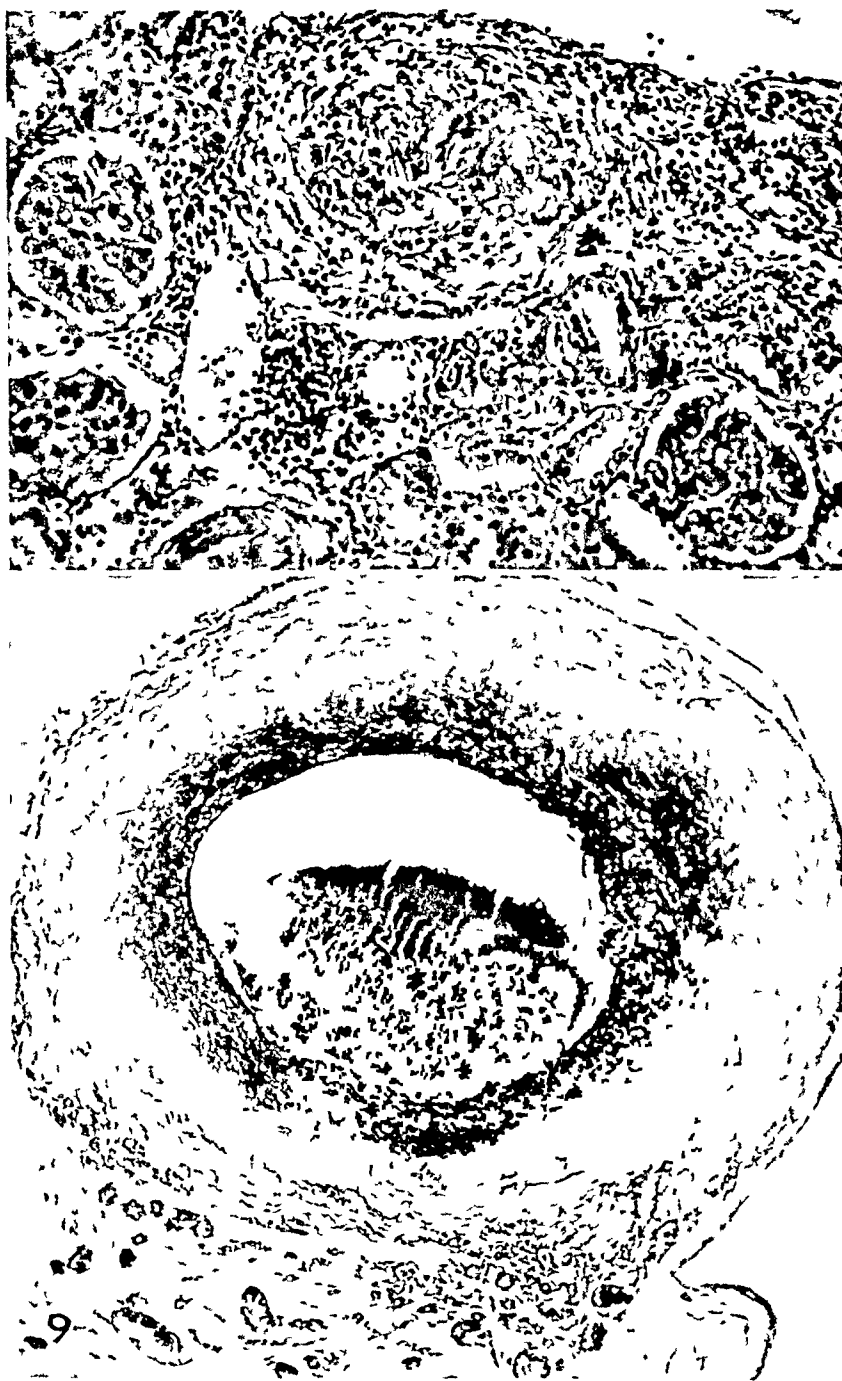


Fig 8 (rat 151) —A branch of a renal artery showing moderately advanced lesions. A small adjacent artery is not involved.

Fig 9 (rat 34a) —Elastic tissue stain of a mesenteric artery. Note the healed lesion with prominent vasorum.

arterial lesions in all stages—developing and healed. Outstanding was the extensive fibrinoid necrosis of the media and subintimal layers, often involving the entire circumference of the wall as a solid ring (fig 3), in many instances, however, it appeared only as a focal area. The endothelial lining was usually intact even

of the branches of the mesenteric vein accompanying the arteries were not involved. The lymphatic vessels were irregularly dilated, somewhat thickened and contained many lymphocytes, attached to the endothelium. No arterial lesions were seen in sections of the lung, the liver, the spleen and the kidneys. In both kidneys

there were calcific deposits in the collecting tubules and hyaline casts in localized areas

**RAT 151**—A young female adult was fed our laboratory diet with 1 per cent (80 mg) of thiouracil daily, beginning Feb 1, 1944. This rat remained active until December 9, the three hundred and twelfth day of thiouracil feeding, then it seemed dull and was killed with chloroform on this day. The leukocyte count was 15,900.

The body weight was 152 Gm. The thyroid gland weighed 0.146 Gm and was dark red. The thymus was atrophic, and there were several slightly enlarged lymph nodes in the area. Both lungs appeared normal throughout. The heart weighed 0.74 Gm. The spleen weighed 0.45 Gm. The liver was grayish purple. The pancreas was unusually white, moderately firm and edematous. The adrenal glands weighed 0.024 Gm and were greenish gray. The kidneys weighed 1.39 Gm, their surfaces were slightly pitted. No calculi were found in the urinary tract. The ovaries were small and contained several very small hyperemic corpora lutea. The pituitary gland weighed 0.009 Gm. The mesentery (fig 4) showed slightly thickened and nodular cords radiating from the base to the intestinal wall, with the mesenteric veins appearing as sharply defined delicate red lines in a normal transparent mesenteric membrane containing fat. The intestinal wall was of normal size and appearance. The lymph nodes at the base of the mesentery and the peripancreatic and perigastric nodes were enlarged and hyperemic, but the inguinal and the axillary nodes were not enlarged.

*Microscopic Examination*—Aside from the changes in the thyroid gland, the adrenal glands, the spleen and the ovaries and the parenchymatous change in the liver and the kidneys, which are considered as characteristic of thiouracil feeding, the special lesions were limited to the arteries of the pancreas, the mesentery, the kidneys and the peripancreatic and perigastric region. These lesions were essentially similar to, but of much less degree and more acute than, those described for rat 100. There were no definite aneurysms, and thrombi were not seen (figs 6, 7 and 8).

**RAT 34a**—A female 7 months old was given our laboratory diet with 1 per cent (80 mg) of thiouracil daily, beginning Jan 29, 1945. Fifteen days later the rat became dull and dyspneic and was killed on the twenty-third day.

The body weight was 240 Gm. The thyroid gland weighed 0.183 Gm, it was dark red and strikingly enlarged for so short a period of thiouracil feeding. The thymus was atrophic, and there were several enlarged nodes in the area. The upper and middle lobes of the right lung and the lower lobe of the left lung were completely consolidated. There were a few scattered areas of consolidation in the lower lobe of the right lung. The upper lobe of the left lung was emphysematous. The heart weighed 1.05 Gm. The spleen was large, the weight, 1.76 Gm. The pancreas was hyperemic, with nodular thickening of the arteries. The liver was grayish purple. The adrenal glands were enlarged, weighing 0.070 Gm. The ovaries contained large bright red corpora. The kidneys weighed 2.77 Gm. There were a few orange-colored calculi in the renal pelvis. The pituitary gland weighed 0.0115 Gm. The mesentery of the small intestine (fig 5) and of the descending colon showed nodular tortuous cords extending from the intestine to the base. Some of the nodules were yellowish but most of them were gray-brown. No vascular lesions were noted in the gastric vessels.

*Microscopic Examination*—No acute lesions were seen in several blocks taken from the pancreas, the mesentery of the small intestine and the colon. The vessel walls were greatly thickened. This thickening was predominantly in the adventitial coat and consisted of a dense new formation of fibrous tissue, in which the vasa vasorum were strikingly enlarged, often congested and possibly increased in number (fig 9). Scattered throughout this thickened adventitia but more frequently in the immediately adjacent areolar tissue were larger and smaller collections of macrophages filled with brownish pigment. Also in the areolar tissue were a few plasma cells and lymphocytes but no granulocytes. Occasionally irregular areas of calcification were seen in the dense hyalinized adventitial coat, but in no vessel was medial or subintimal calcification noted. The media was not sharply outlined either on the intimal or on the adventitial side. No inner elastic layer could be made out, and while some filaments of elastic tissue were seen in the outer zone of the media, the sharp line of demarcation between media and adventitia was lost because of the extensive fibrous tissue replacement of the media.

#### COMMENT

Three rats (2 females and 1 male) showing typical lesions of periarteritis nodosa were detected in a group of more than 100 rats kept under the same laboratory conditions and given thiouracil. In 2 of the 3 rats this pathologic change presumably developed toward the end of the long period of thiouracil feeding, although in 1 (100) there was abundant evidence of chronicity (healing and healed lesions). The third rat had received thiouracil for twenty-two days, and the lesions were largely healed. The lesions occurred chiefly in the branches of the superior and the inferior mesenteric arteries, and in rat 151, also in branches of the renal arteries. The regional lymph nodes were enlarged and hyperemic. No arterial lesions were found in the spleen, the liver, the heart, the lungs, the thyroid gland, the gonads or the intestines.

This distribution of the lesions was in the path of absorption from the gastrointestinal tract and may be significant, particularly if sensitization is in some way dependent on antigenic substances spreading from the perivascular lymphatics as Klotz<sup>18</sup> has suggested. Rats 100 and 34a had extensive chronic and acute pneumonia, while rat 151 showed no pneumonic lesions or other signs of infection. These rats had not been used for any previous experimental procedures, and we have no clue as to why this manifestation of hypersensitivity developed in only 3 rats. The question of whether thiouracil could be a factor is raised. Such an association does not seem likely since rat 34a had received the drug for twenty-two days and only the healed arterial deformities (hyalinization of the media, fibrosis and calcifi-



cation in the adventitia) were present. However, one does not know how rapidly healing of the lesions can occur. If the disease in rats is similar to human periarteritis nodosa, the lesions may develop in a few days (Rich). The very high percentage (97) reported by Wilens and Spioul showed a more extensive distribution of lesions than was found by us. They suggested that possibly dietary factors may have influenced the incidence since only in 1 of 75 rats receiving meat and vegetables did the lesions occur while 46 of 356 rats whose diet lacked one or the other of these ingredients had the lesions. The cases reported by Wilens and Spioul occurred in old rats—mostly over 700 days of age—while the oldest of our 3 rats was less than 500 days old.

The patient with periarteritis nodosa whose case was reported by Rich and referred to in the foregoing paragraph received sulfathiazole on two occasions, which might be looked on as providing the sensitizing and testing doses, while our rats received the thiouracil continuously. Many authors<sup>19</sup> have warned against using the same sulfonamide drug when it is necessary to repeat the course of treatment since the various "toxic" reactions occur in a higher percentage of such cases.

In view of the work of Schonholzer,<sup>20</sup> Wedum<sup>21</sup> and others it seems possible that hypersensitivity was induced by specific thiouracil-protein conjugates from proteins or their hydrolytic products in the intestine, which were absorbed by way of the lymphatics and thus reached the arterial walls. The normal rat is difficult to sensitize with any antigen, but if

adrenalectomized,<sup>22</sup> it is fairly easily sensitized to horse serum.

If the periarteritis nodosa-like lesions observed are allergic reactions, it is possible that the hypertrophy of the adrenal medulla and the severe injury of the cortex which we have reported<sup>23</sup> following prolonged thiouracil feeding in rats may have rendered them susceptible to sensitization similar to that which can be induced following adrenalectomy. Lesions morphologically similar to those of periarteritis nodosa in man are of fairly common occurrence in white rats. If these lesions are manifestations of hypersensitivity, the high incidence is somewhat paradoxical.

#### SUMMARY

Periarteritis nodosa developed in 3 rats, 1 male and 2 females, reared in the laboratory, during continuous feeding with 0.5 per cent and 1 per cent thiouracil. About 100 other rats of the same stock and age fed with thiouracil did not show such lesions. The lesions were confined to the mesentery of the small intestine, the colon, the pancreas, the perigastric and peripancratic region and the kidneys of 1 animal (151). Two of the rats had extensive chronic and acute pneumonia, the third had no signs of infection. Neither rat had been previously used. All 3 rats showed approximately the same changes in the thyroid gland, the spleen, the gonads, the liver, the kidneys and the adrenal glands that are associated with thiouracil poisoning.

22 Flashman, D. H. *J. Infect. Dis.* 38:461, 1926.

23 Marine, D., and Baumann, E. J. Hypertrophy of the Adrenal Medulla of White Rats in Chronic Thiouracil Poisoning, *Am. J. Physiol.*, to be published.  
Baumann, E. J., and Marine, D. Involution of the Adrenal Cortex of Rats Fed with Thiouracil, *Endocrinology*, to be published.

19 Dowling, H. F., and Lepper, M. H. *Am. J. M. Sc.* 207:349, 1944.

20 Schonholzer, G. *Klin. Wchnschr.* 19:790, 1940.

21 Wedum, A. G. *J. Infect. Dis.* 70:173, 1942.

# Case Reports

## LATENT PRIMARY CARCINOMA OF THE THYROID GLAND

NATHAN MITCHELL M D, BROOKLYN

A mass in the neck is usually the initial finding in most cases of cancer of the thyroid gland. In a small percentage of cases the first symptoms may be caused by precocious metastases in remote viscera. As Willis<sup>1</sup> pointed out, in the large majority of instances latent cancer of the thyroid becomes manifest through deposits in the bones. In such cases the diagnosis of metastatic cancer of the thyroid gland is often made only after surgical operation on a limb and subsequent histologic examination of the osseous tumor have identified the thyroid origin. Willis also recorded from the literature hepatic, intestinal and cerebral metastases that were operated on and later proved to be secondary deposits from latent cancer of the thyroid gland. No instance in which pulmonary metastases from a primary tumor of the thyroid gland giving rise to the clinical picture in the case now reported has been found described in the available literature.

### REPORT OF A CASE

The patient was a 51 year old white woman who first became ill in the summer of 1943. At that time she noted gradually increasing fatigability, anorexia and loss of weight. Several months later cough appeared with expectoration of whitish mucoid sputum which was occasionally blood streaked. Two months before admission to the Mount Morris Tuberculosis Hospital she noted marked dyspnea, which had become increasingly severe. A roentgenogram of the chest in June 1944 showed numerous nodular and linear densities scattered throughout both lung fields. She was admitted on July 7, 1944.

There were signs of scattered areas of pulmonary consolidation, moderate exophthalmos and moderate emaciation. No masses were felt in the neck. The white blood cell count was 14,200, with 84 per cent polymorphonuclear leukocytes, 4 per cent monocytes and 12 per cent lymphocytes. The red blood cell count was 5,000,000, with a hemoglobin content of 16.5 Gm per hundred cubic centimeters (116 per cent). The sputum was negative for tubercle bacilli on three occasions. Roentgenograms of the chest revealed irregularly shaped and variably sized nodular and linear densities in the right lung, particularly below the level of the fourth anterior rib. In the left lung densities similar to those seen in the right lung were found between the levels of the first and third ribs anteriorly. Throughout both lungs there were irregularly outlined areas of radiolucency which were suggestive of small areas of cavitation. Comparison of this roentgenogram with that of June 1944 showed definite increase in infiltration.

From the Mount Morris Tuberculosis Hospital, Tuberculosis Division, New York State Department of Health

1 Willis, R. A. *The Spread of Tumors in the Human Body*, London: I & A Churchill, Ltd, 1934

throughout both lungs. Despite vigorous supportive therapy, the patient failed rapidly and died after six days in the hospital.

*Autopsy* (one hour after death)—There was moderate emaciation. The thyroid gland was not palpable. No lymphadenopathy was apparent externally. The breasts were small and atrophic, and no masses were felt within the mammary parenchyma.

The left pleural cavity contained 500 cc and the right pleural cavity 750 cc of a clear straw-colored fluid. There were about 150 cc of a similar fluid in the pericardial sac. No enlarged lymph nodes were seen in the mediastinum.

The heart was not enlarged. Several indurated, slightly elevated nodules 0.1 cm in diameter were present beneath the visceral layer of the pericardium near the base of the heart. No tumor nodules were seen in the myocardium.

The left and right lungs, although not enlarged, weighed 840 and 960 Gm. There were no adhesions in either pleural cavity. Minute yellow-gray streaks were noted beneath the visceral pleura of both lungs. On section innumerable rounded and irregularly oval granular opaque gray-white nodules were seen scattered throughout both lungs from apex to base (fig 1). The nodules were extremely firm. Many of them appeared to be formed by confluence of smaller foci. Where the pulmonary parenchyma was visible, tiny nodules measuring less than 0.1 cm were noted. At the base of the right lower lobe was a roughly quadrangular focus of dark red tissue measuring 4 by 2 by 2 cm, slightly elevated above the level of the surrounding tumor-bearing pulmonary parenchyma. The major bronchi showed no areas of ulceration and contained no tumors. There were no thrombi in the larger branches of the pulmonary arteries.

On the anterior border and surface of the spleen were numerous thin-walled cystic structures measuring from 0.1 to 0.5 cm in diameter. On section these appeared to be limited to the capsule. They contained a small amount of homogeneous yellow-brown material.

All lobes of the liver were occupied by numerous rounded and irregular foci of granular opaque gray-white tissue ranging in size from 0.3 to 4 cm. The nodules were well demarcated from the brown-red hepatic parenchyma. Occasionally, grayish streaks could be seen extending into the sinusoids. No gross invasion by tumor tissue was seen in either the portal or the hepatic venous channels.

The gallbladder wall was slightly thickened and contained about 50 calculi, faceted and dark green. The extrahepatic bile ducts were patent.

The kidneys contained several small nodules in the cortex, for the most part directly beneath the capsule.

The cervix uteri was indurated, and a small red polyp protruded into the endocervical canal. The endometrium was smooth and glistening throughout. A small myomatous nodule was present in the myometrium. A few tiny cystic structures were noted beneath the serosa covering the posterior surface of the uterus. In

the right broad ligament was a thin-walled cystic mass measuring 3 cm in diameter. It was attached to the region of the fallopian tube by a thin stalk.

The thyroid gland was not increased in size, but a firm mass was felt in the right lobe. The left lobe showed no abnormalities. On section of the right lobe

a rounded mass measuring 1.25 cm in diameter was noted in the anterolateral aspect of the lobe (fig 2). The mass was opaque and gray-white, with prominent centrally placed hemorrhagic streaks and yellow areas of necrosis. The nodule was depressed below the level of the brown thyroid parenchyma. The capsule was



Fig 1—Cut surface of the left lung showing minute rounded and larger irregular confluent nodules of metastatic cancer,  $\times 0.5$

Fig 2—Both lobes of the thyroid gland sectioned in the horizontal plane. The left lobe contains no tumor tissue. The cancerous mass in the anterolateral aspect of the right lobe has invaded a small portion of the capsule of the gland. In the center of the mass hemorrhage and necrosis are prominent,  $\times 1.4$

Fig 3—A section through the myometrium showing the uterine venules filled with solid plugs of cancerous tissue in which papillation is prominent. Section of paraffin-embedded tissue stained with hematoxylin and eosin,  $\times 120$

invaded by the tumor tissue in one small area. No obvious involvement of regional lymph nodes and no vascular invasion were seen on gross examination.

A sagittal section through the lumbar vertebrae revealed extensive replacement of the hemopoietic tissue by opaque gray-white almost bony hard tissue.

*Microscopic Observations*—The most prominent histologic change in many of the viscera was extensive intravascular proliferation of tumor cells. In the heart, groups of epithelial cells were noted in an occasional myocardial venule. The epicardial venules and the vasa vasorum of the aorta were filled with solid plugs of tumor tissue in papillary formation. The interlobar and interlobular veins of the pancreas contained cancer cells with an alveolar pattern. In the liver many parenchymal metastases were present. The tumor tissue extended into the walls and lumens of the portal and hepatic venous radicles. Tiny nodules of metastatic cancer were present in the adrenal cortex, the capsular venules contained small tumor emboli. Although several metastatic nodules were observed in the renal cortex, no definite tumor cells were seen within the renal vessels. The pelvic viscera showed the most widespread and extensive permeation of veins by the cancerous tissue. In the uterus particularly, almost every venule and many of the lymphatic channels contained solid masses of cancerous epithelial cells (fig 3). The ovarian venules were similarly involved by papillary tumor tissue.

The small trabecular veins of the spleen contained occasional epithelial cells similar to those in other vessels. No true metastatic foci were found in the splenic parenchyma. A multilocular lymphangioma was present in the capsule of the spleen and corresponded to the cystic structures noted grossly. The cysts were lined by either flattened endothelium-like cells or low cuboidal cells. The lumens of the cystic spaces were occupied by very pale eosinophilic homogeneous material containing a few histiocytic cells. Adjacent to and within some of the cysts were small nodules of papillary neoplastic tissue identical with many of the foci noted in other organs.

Isolated groups of carcinomatous cells were found in the venous sinusoids of the vertebral marrow. Prominent new bone formation was seen alternating with cancer cells within the marrow. Rows of osteoblastic cells were present at the periphery of many of the new spicules of bone, and it was difficult to dissociate them from the cancerous epithelium. Many of the solid neoplastic foci showed prominent papillary formation. No actual destruction of the bony trabeculae of the marrow was seen.

Little intact parenchyma remained in the lungs. Many sections showed the bulk of the pulmonary tissue to be occupied by papillary epithelial tumor tissue. Tiny discrete and larger confluent foci were scattered throughout. In the smaller nodules the tumor occupied the lumens of the alveoli, and the interalveolar septums were intact. In the larger confluent foci the interalveolar walls were poorly delineated because of compression by the tumor cells. In some situations necrotic and necrobiotic changes were noted. Densely basophilic oval bodies composed of finely laminated amorphous material and resembling the so-called corpora amylacea were observed both in the necrotic foci and in places where the cells were intact (fig 4). Calcium could be demonstrated in a few of these structures by the von Kossa stain. Extensive permeation of the blood vessels and the lymphatic channels by the cancerous tissue was evident. The pulmonary veins were predominantly involved. The lumens of these vessels were filled with solid clumps of cancer cells. Some

of the larger pulmonary arteries contained recently formed thrombi in which could be seen large collections of cancer cells, some of which were arranged in glandular formation. Some of the bronchial arterial branches were filled with small plugs of tumor tissue. The perivascular and peribronchial lymphatic vessels, as well as the subpleural lymphatic channels, were filled with small tumor thrombi.

The left lobe of the thyroid gland contained no tumor tissue. In the right lobe the rounded nodule seen grossly, which was obviously carcinomatous, was flanked by a small papillary adenocystoma on one side (fig 5) and a colloid adenomatous nodule on the other.

In contrast with the normal neighboring thyroid parenchyma, the alveoli in the cancerous nodule were placed more closely, and there was almost complete disappearance of the interacinous connective tissue. In addition the cells comprising the alveoli within the cancerous nodule were larger, distinctly hyperchromatic and variable in size and shape (fig 6). In some acini there was a moderate amount of colloid, while in others the colloid was scanty or absent. Active growth could be identified in some of the thyroid follicles by the presence of small papillations composed of epithelial cells. In other alveoli this tendency was more pronounced and solid plugs of epithelial cells were seen within the lumens. In places the cancer cells were growing in cords and clumps without any attempt at gland formation. Clumps of cancerous cells which were markedly hyperchromatic extended beyond the limits of the roughly rounded cancer nodule. The capsule of the thyroid gland was invaded at one point, and clumps of cancer cells were seen to extend between the normal thyroid acini of the regional lobules. Prominent venular invasion by small groups of epithelial cells was noted. Solid clumps of epithelial cells were present in the lumens of these vessels, and occasionally the endothelium had been replaced by a single row of neoplastic elements. Extensive hemorrhage and necrosis were present in the center of the cancerous nodule. A large amount of fibrous tissue was also centrally located and was composed of broad bands of afibrillar, densely eosinophilic homogeneous collagen in which there was only an occasional fibroblastic cell. In some places the collagenous tissue was entirely acellular.

The papillary adenocystoma, which measured approximately 0.3 cm in diameter, was rimmed peripherally by a thin layer of fibrous connective tissue on which rested a single layer of cuboidal epithelium. In the center the papillary projections were seen in cross section and appeared as rounded alveolar structures with a central core of slightly vascularized connective tissue. The epithelial cells were regular in size and shape, and no atypical proliferation was visible. A moderate amount of colloid was seen in the alveoli and in the lumen of the cyst between the papillary projections and the cyst wall. The colloid adenomatous nodule approximated the dimensions of the adenocystoma described and was composed of various-sized acini with abundant colloid in the center of the glands. The epithelial linings were made up of flattened cuboidal cells.

*Anatomic Diagnosis*—Papillary adenocarcinoma of the right lobe of the thyroid gland with metastases to the lungs, visceral pleura, visceral pericardium, liver, kidneys, lumbar vertebrae, splenic capsule and adrenal glands, neoplastic embolism of myocardial, splenic, pancreatic, ovarian and myometrial venules, hydrothorax, hydropericardium, recent hemorrhagic infarct of the lower lobe of the right lung, multilocular lymphan-

gioma of the splenic capsule, fibrolipoma of the kidney, hydatid of Morgagni of the right broad ligament serosal cysts of the uterus, small leiomyoma of the uterus, adenomatous polyp of the cervix, chronic cholecystitis and cholelithiasis, old tubercles of the spleen

It was stressed that the term "latent" should be applied to any cancer the presence of which was made known by metastatic deposits in remote viscera before any symptoms or signs refer-

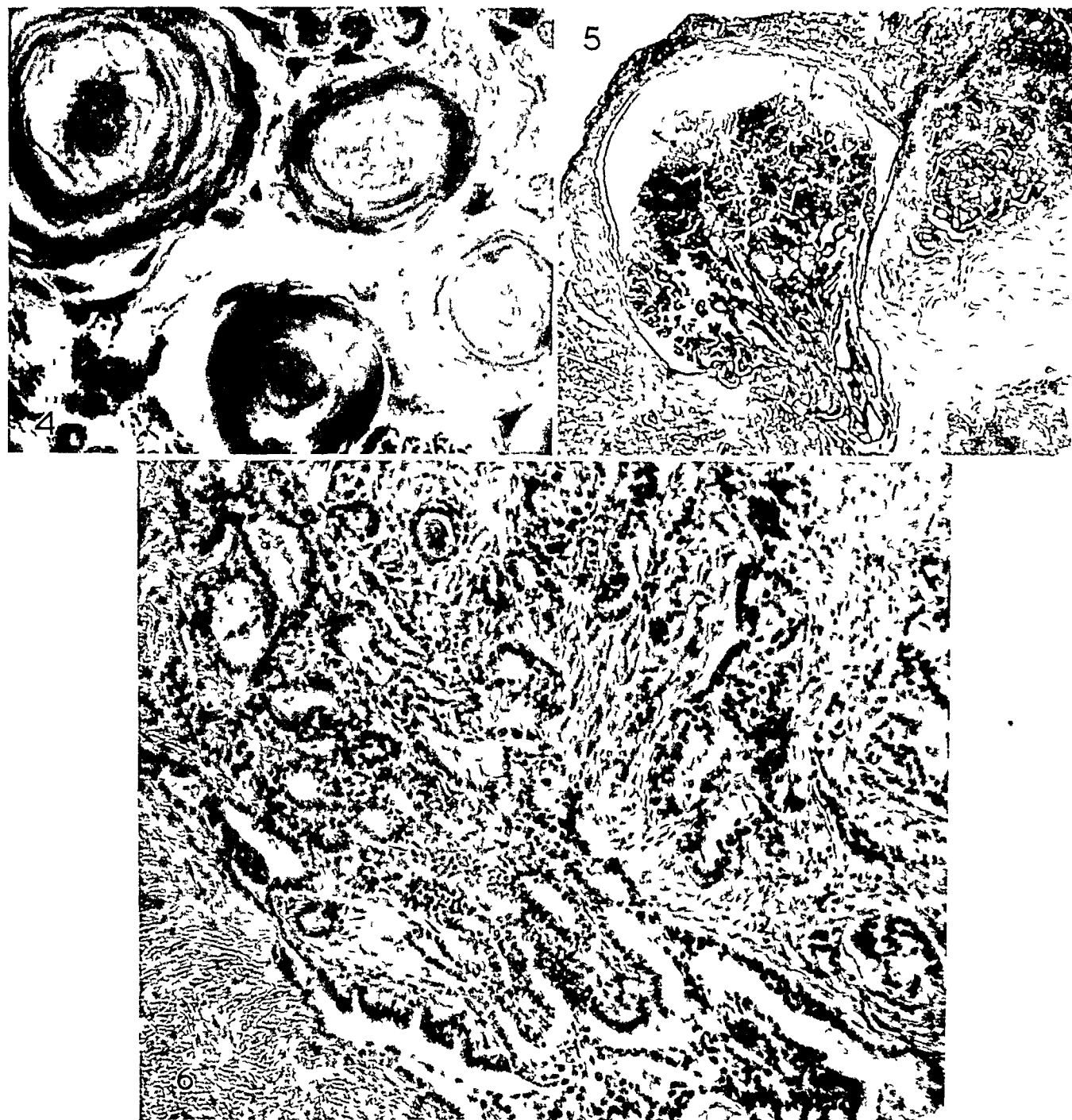


Fig 4—Densely basophilic oval bodies composed of finely laminated amorphous material found in a portion of one of the pulmonary metastases in which necrosis was not prominent. Shadow forms of epithelial nuclei are present in the centers of some of the bodies. Section of paraffin-embedded tissue stained with hematoxylin and eosin,  $\times 590$ .

Fig 5—A hyperplastic papillary adenocystoma is situated between the cancerous mass at the right and the normal thyroid acini at the left. Necrosis and hyaline fibrosis may be noted within the cancerous nodule. Section of paraffin-embedded tissue stained with hematoxylin and eosin,  $\times 14$ .

Fig 6—The cells comprising the thyroid acini within the cancer nodule are hyperchromatic, and atypical proliferation is seen.

#### COMMENT

A precise definition of latent primary carcinoma following that proposed by Willis has been offered by Gewanter, Mitchell and Angrist.<sup>2</sup>

able to the site of the cancer's origin had appeared. The cancer now described seems to fit

<sup>2</sup> Gewanter, A. P., Mitchell, N., and Angrist, A. *Arch. Path.* **35**: 66, 1943.

into that category. The thyroid mass was neither palpable nor visible, nor did it occasion any symptoms. Suggestive exophthalmos was noted when the patient was admitted to the hospital, but the probability exists that this isolated sign was caused by the physiologic activity of the widespread metastases. In this connection it is interesting to speculate about the significance of the densely basophilic oval bodies which were found in some of the metastatic nodules, for such concretions, which were found intimately associated with degenerative and necrotic changes in the cancerous epithelium, may represent some form of altered thyroid secretion. Exophthalmos had not been noted before admission, and there were no other symptoms of overactivity of the thyroid gland.

Inaccessibility and prominent vascular invasion both contributed in making this tumor a latent one. Despite the superficial location of the cancer in the right lobe of the thyroid gland, its small size and its failure to elevate the capsule in any one place precluded clinical discovery of the mass. The ribbon muscles of the neck effectively hid the small tumor from the examining fingers. Equal importance must be attributed to the early and extensive growth of the cancer cells within the venules of the thyroid gland. The initial symptoms were referable to the lungs, and this attests well to the fact that the pulmonary metastases were established early in the course of the disease and grew rapidly while the primary growth was relatively quiescent. The tendency of these particular cancer cells to penetrate vascular channels is well shown in the metastatic foci in the lungs and the liver. The pulmonary veins were extensively involved by tumor thrombi, and this afforded adequate opportunity for further dissemination of the tumor to viscera in the path of the systemic circulation. In turn, the invasion of the large hepatic veins by metastatic nodules in the liver was another prolific source of pulmonary metastases. The permeation of the branches of the bronchial arteries by microscopic tumor emboli provided yet another mechanism of pulmonary seeding.

The prominent involvement of the lungs and the tendency of the pulmonary metastases to line the alveolar walls simulate the appearance described for primary alveolar cancer of the lung. From the observations in this and other cases of small latent primary carcinoma of the thyroid and the prostate gland, the diagnosis of primary alveolar cancer of the lung should be made only after careful search for all such possible distant foci of origin.

The genesis of the diffusely scattered venous permeation in various organs is open to question. That the cancerous intravascular growths do not represent tertiary venous invasion is suggested

by the absence of discrete metastatic nodules in the organs affected. It is difficult to visualize such widespread involvement of the ovarian and myometrial venules from simple postmortem manipulation of the viscera. Retrograde venous embolism must be considered despite the absence of gross occlusion of the larger veins (which is stressed by Willis), because of the widespread obstruction of the venous channels in the pulmonary and portal beds. Finally consideration must be given to permeation by extensive contiguous growth within the venous channels from undisclosed arterial emboli.

It has been said that in 80 to 90 per cent of all cases of carcinoma of the thyroid gland the carcinoma arises from preexisting adenoma<sup>3</sup> and that "usually the development of the adenocarcinoma has destroyed all trace of the pre-existing adenoma and this original nidus can be recognized only from the history given by the patient." Despite the absence of such a history in this case, there is histologic evidence of preexisting adenoma. Adjacent to the cancerous nodule there were a well developed benign papillary adenocystoma and a colloid adenomatous nodule. The wide bands of deeply eosinophilic homogeneous acellular fibrous tissue in the large cancerous nodule also indicate that another lesion of many years' duration must have been present.

Histologically, this cancer of the thyroid fits into group 2 of the classification proposed by Lahey, Hare and Warren<sup>3</sup> and others<sup>4</sup>. While there was considerable anaplasia in the primary tumor as well as in some of the metastases, the bulk of the tumor showed prominent papillary formation.

#### SUMMARY

In the case of latent primary carcinoma of the thyroid gland presented, there is evidence that the cancer arose at the site of an adenoma of long standing. The presenting clinical picture was one of pulmonary involvement and was caused by early and prolific metastases in the lungs.

The small size of the primary tumor, without distortion of the gland, the invasion of the venules of the gland by the cancer cells and the rapid growth of the metastatic foci in the lungs account for the latency in this case.

A prominent feature of the tumor was its widespread permeation of veins throughout all the areas of involvement.

The gross and microscopic pulmonary changes bore a resemblance to primary alveolar cancer of the lung.

3 Lahey, F. H., Hare, H. F., and Warren, S. *Ann Surg* **112** 977, 1940.

4 McClintock, J. C., Clinck, G. H., Jr., and Conrad, J. E. *Surg, Gynec & Obst* **72** 150, 1941.



# ADRENAL CORTICAL ADENOMA OF THE EPIDIDYMIS

ANN FREEMAN, M D, CHICAGO

Nodules of tissue repeating the structure of cortical adenoma of the adrenal gland and displaced along the course of the wolffian duct or its adult derivative structures have been described frequently. In the publications reviewed by Nelson,<sup>1</sup> Glynn<sup>2</sup> and others these nodules are described as found near the adrenal gland, beneath the capsules of the kidney and the liver along the course of the ovarian artery and vein, in the broad ligament of the ovary and in the ovary itself, along the course of the spermatic artery and vein and in the connective tissue of the inguinal canal. However, only a few nodules have been described which occurred in the region of the testis or the epididymis in man.

Dagonet<sup>3</sup> described 2 nodules of adrenal cortical tissue, 1 on the right spermatic vein and 1 between the right epididymis and the adjacent tubules of the corresponding testis in an infant aged 21 days. Micheal<sup>4</sup> recorded 7 cortical adenoma nodules occurring between the testicular tubules and the epididymal ducts, 4 occurred in adults and 3 in infants. Two heterotopic nodules were near the right epididymis in one infant, and 1 was near the left epididymis in another. Wiesel<sup>5</sup> reported the examination of 15 pairs of testicles and epididymides from newborn infants and an equal number from males whose ages ranged between 1 and 60 years. He found cortical adrenal gland tissues repeatedly near the testis and the epididymis in specimens from the newborn but only 2 adenomas among the tubules of the epididymis. Kirkbride<sup>6</sup> examined 54 tissues and reported 8 small adrenal cortical adenomas in the region of the testis and the epididymis. Berger,<sup>7</sup> R Meyer,<sup>8</sup> Panā,<sup>9</sup> Pilliet, Regaud and Loisel, according to Glynn,<sup>2</sup> Roth, according to Ulrich,<sup>10</sup> Monserrat

and Latienda,<sup>11</sup> and others observed similar nodules of adrenal cortical tissue in this region. Although the number of these heterotopic cortical adenomas reported in newborn and other male infants is small, the number observed in this region in adults is much less.

Jaffe<sup>12</sup> stated that accessory nodules disappear with advancing age because they are physiologically unnecessary in the presence of adequate normal adrenal glands. Others agree that most of these rests disappear before puberty. Wiesel<sup>5</sup> found cell masses and strands resembling cortical tissues near the epididymis in tissues from youths but none fully developed in the epididymis in tissues from adults. Micheal reported cortical tissues among the tubules of the right epididymis in 4 adults. Marsella,<sup>13</sup> in 1934, described a nodule among these ducts of the epididymis in a man aged 34 years, and Nelson<sup>1</sup> reported nodules in the region of the testis and the epididymis in 6 men. The tissues which Nelson examined were either within or just outside the tunica albuginea and either behind or just above or below the rete testis. His report discussed the individual characteristics in detail. All of these accounts of heterotopic tissue in newborn infants and in adults have the microscopic structure of the glomerular and fascicular zones of the adrenal gland.

## REPORT OF A CASE

A white man aged 73 years entered St Luke's Hospital, where routine examinations established a diagnosis of glioma of the temporal lobe of the right cerebral hemisphere. The postmortem examination, one month later, confirmed this diagnosis. No heterotopic adrenal gland tissues were observed grossly in the viscera, and these included the adult derivative structures of the wolffian ducts. Each adrenal gland weighed 10 Gm and was not unusual. Histologic examination of the testes showed the usual characteristics. In the sections from one including the epididymis, the rete testis and the testicular tubules, a nodule of adrenal cortical tissue, 4 by 1 mm in its external dimensions, was observed in the connective tissue outside the tunica albuginea posteriorly, at a level which corresponded to the level of the rete testis and between the tubules of the epididymis. This tissue was partially encapsulated. The cells (*A* and *B* in the figure) corresponded in structure and arrangement to the glomerular and fascicular zones

From the Henry Baird Favill Laboratory, St Luke's Hospital

This study was aided by the Winfield Pack Memorial Fund

- 1 Nelson, A. A. *Arch Path* **27** 955, 1939
- 2 Glynn, E. E. *J Obst & Gynaec Brit Emp* **28** 23, 1921
- 3 Dagonet, J. *Ztschr f Heilk* **6** 1, 1885
- 4 Micheal, J. *Deutsches Arch f klin Med* **43** 120, 1888
- 5 Wiesel, J. *Wien klin Wchnschr* **11** 443, 1898
- 6 Kirkbride, M. B. *Arch f Entwcklungsmechn d Organ* **32** 717, 1911
- 7 Berger, L. *Arch d'anat micr* **32** 315, 1936
- 8 Meyer, R. *Ztschr f Geburtsh u Gynak* **71** 221, 1912
- 9 Panā, C. *Minerva med* **1** 76, 1931

10 Ulrich, A. *Beitr z path Anat u z allg Path* **18** 589, 1895

11 Monserrat, J. L., and Latienda, R. I. *Arch Soc argent de anat norm v pat* **5** 367, 1943

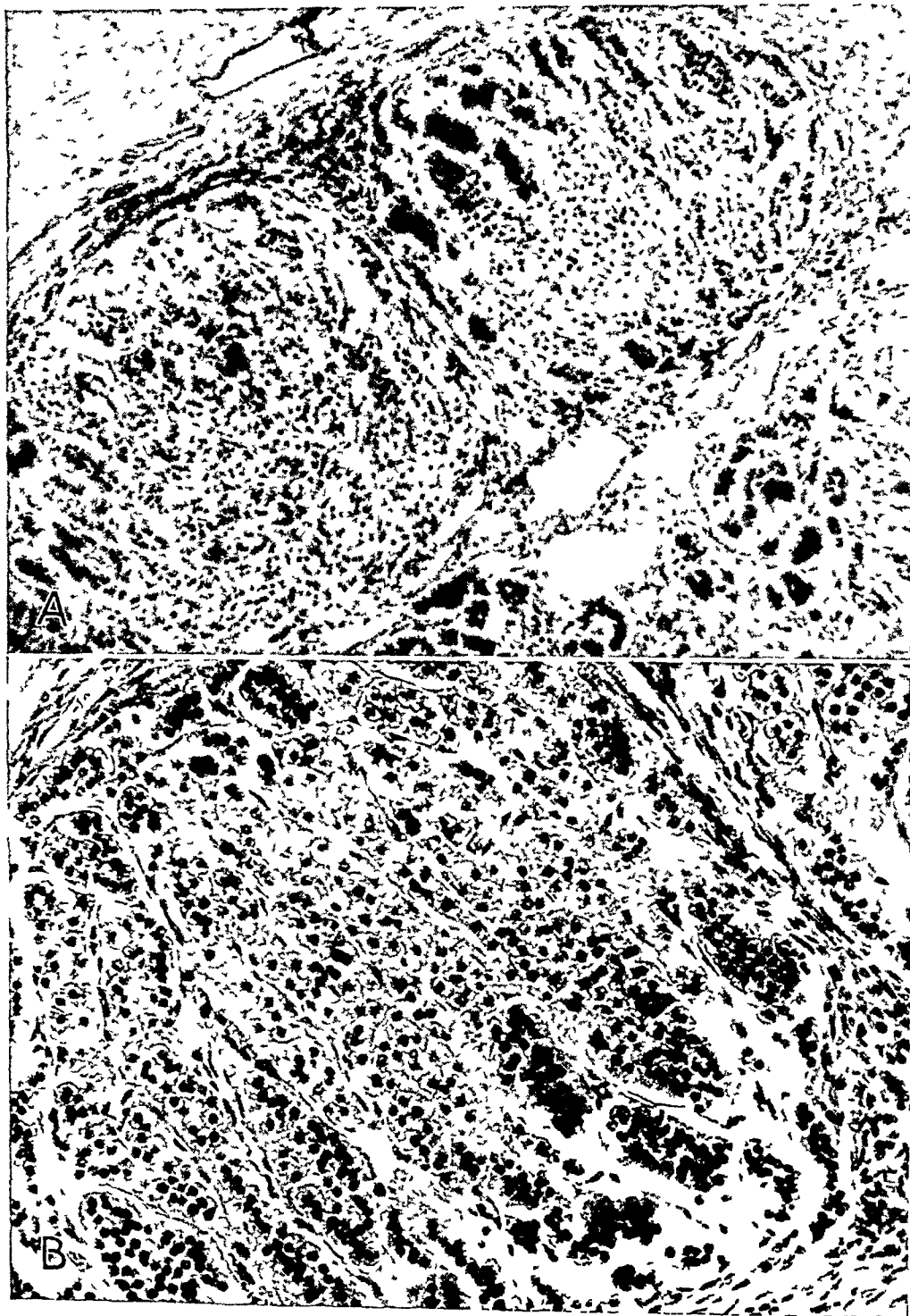
12 Jaffe, H. L. *Arch Path* **3** 414, 1927

13 Marsella, A. *Arch ital di urol* **11** 281, 1934



of the adrenal gland. The cords of cells opposite to those partially encapsulated were in rows separated from each other by strands of fibrous connective tissue. This histologic structure was identical with that characterizing the cells of cortical tissue. Cells of the reticular zone, as well as aggregates of medullary tissues of the adrenal gland, were absent.

located in the region of the testis and the epididymis. The information available is not sufficient to establish the incidence of these nodules in newborn infants, older infants or adults. They seem to be less frequent in adults. Perhaps accessory nodules atrophy with age in the pres-



*A*, photomicrograph (low power) illustrating the nodule of heterotopic adrenal gland tissue in the epididymis. Two small ducts are at the upper edge. *B*, photomicrograph illustrating the zonal arrangement and vacuolated cytoplasm of the cells ( $\times 198$ ).

#### SUMMARY

Nodules of tissue repeating the structure of cortical adenoma of the adrenal gland and displaced along the course of the wolffian duct or its adult derivative structures have been described frequently. Of these, only a few nodules oc-

curred in the region of the testis and the epididymis. The information available is not sufficient to establish the incidence of these nodules in newborn infants, older infants or adults. They seem to be less frequent in adults. Perhaps accessory nodules atrophy with age in the pres-

# BRONCHIAL ASTHMA AND TUBERCULOSIS

PETER A. HERBUT, M.D., PHILADELPHIA

That bronchial asthma and pulmonary tuberculosis can occur in the same patient at the same time is today a well established fact. All recent and some not so recent publications deal with the subject from a purely clinical approach. Some authors studied the incidence of tuberculosis in asthmatic patients,<sup>1</sup> whereas some reported the incidence of asthma in tuberculous patients,<sup>2</sup> and others still made no statistical studies but simply described the two diseases as occurring concomitantly in the same patients.<sup>3</sup> Since the incidence of asthma in patients with pulmonary tuberculosis is given as only 0.25 per cent to 3.1 per cent<sup>2</sup> and the incidence of tuberculosis in asthmatic patients as 4.4 per cent,<sup>1a</sup> 6 per cent,<sup>1b</sup> 8 per cent<sup>1c</sup> and 10 per cent,<sup>1a</sup> the number of deaths must be relatively few and the number of postmortem examinations fewer still. At any rate, in none of the more recent publications is there any mention of histopathologic observations of the tracheobronchial tree in fatal cases of bronchial asthma associated with tuberculosis, and neither in these reports nor in a search of the medical literature have I been able to find any references to such studies. It is thus not apparent whether the two processes exist as independent and unrelated lesions or whether they are intimately intermingled. Because of the paucity of information on this point the following report of a case is deemed worthy of publication.

## REPORT OF A CASE

A white woman 50 years old first began to have asthma at the age of 46 and for the last four years before her death had increasingly more severe and more frequent asthmatic seizures. At first these were easily controlled by injections of epinephrine hydrochloride, but later this drug was less effective. Her last attack started a week prior to her admission to the Jefferson Medical College Hospital, and because administration of both epinephrine and theophylline ethylenediamine did not alleviate her distress she was hospitalized. She was markedly dyspneic, had wheezes over her entire chest and was semicomatose. Caffeine and sodium benzoate U. S. P., epinephrine, theophylline ethylenediamine and oxygen, which was given continuously, were of no avail, and she died of suffocation twenty-four hours after admission.

From the Clinical Laboratories, Jefferson Medical College Hospital.

1 Harkavy, J., and Hebal, S. *Am Rev Tuberc* **21** 644, 1930. Sterling, A. *J Allergy* **1** 185, 1929-1930. Laub, S. *J Illinois M J* **62** 54, 1932. Fraenkel, E. M. *Brit M J* **2** 513, 1934.

2 Locker, A. M., and Davidson, A. G. *J Allergy* **15** 108, 1944.

3 Giffin, H. Z. *Am J M Sc* **142** 869, 1911.

Röntgenograms of the chest were not made, and the only significant laboratory findings were a leukocyte count of 28,200 cells per cubic millimeter with 90 per cent polymorphonuclear leukocytes and 2 per cent eosinophils, a heavy cloud of albumin in the urine, the presence of acetone and occasional pus cells and granular casts in the urine, and a specific gravity of 1.038. Although cutaneous tests for sensitivity to the common allergens were performed sometime before the last illness and were said to be positive, the record does not contain a list of the substances to which the patient was sensitive.

Necropsy, twelve hours after death, revealed the lungs to be voluminous, they filled the pleural cavities entirely and encroached on the mediastinum anteriorly. Their serosal surfaces were mottled light gray and slate gray and entirely free of adhesions. The lungs were light and feathery, weighing 220 and 460 Gm respectively. Crepitations were fine and somewhat decreased. Cut surfaces disclosed a soft pinkish gray, rather dry parenchyma wherein the medium-sized bronchi and their subdivisions were conspicuous because, although of normal caliber, they were filled to capacity with thick, sticky, tenacious, light gray, mucoid material that pulled away in long strings. The mucosa of the large bronchi and the trachea was slightly congested, and the lumens contained less mucus than did those of the more distal portions of the bronchial tree. Immediately beneath the pleura and just lateral to the apex of the upper lobe of the left lung there was a firm, sharply circumscribed but not encapsulated nodule of fibrous tissue that measured approximately 1 cm in greatest diameter. Grossly it contained no evident areas of softening nor did it reveal any tubercles at its periphery. There were no other fibrous or miliary foci in either lung. The mediastinal lymph nodes were anthracotic but otherwise normal. The heart weighed 300 Gm and the right side showed no hypertrophy. In none of the thoracic or abdominal organs was there any evidence of active tuberculosis. In each maxillary sinus the lining membrane was thickened to as much as 2 mm across. The nasal mucosa was only moderately thickened. An attempt was made to secure the lining of the sphenoid and ethmoid sinuses through the nose but this was unsuccessful. The brain was not examined.

Routine sections were made of all the thoracic and abdominal organs and in addition numerous sections of the left lung because it was available for reexamination. The lining of the trachea and larger bronchi to an internal diameter of approximately 6 mm disclosed only those changes typical of bronchial asthma (fig 1). The lumen contained a varied amount of mucus and debris. The trachea was either partially denuded of its lining epithelial cells or showed islands of fairly normal-appearing cells containing occasional droplets of mucus and in scattered areas covered with cilia. The bronchi showed more denudation, more secretion and a complete absence of cilia. The basement membrane throughout was broad, dense and hyaline. The submucosa showed considerable edema, congestion of the capillaries and a diffuse infiltration of polymorphonuclear leukocytes, plasma cells and lymphocytes. Eosinophils were rather sparse in the trachea but became more numerous as the smaller bronchi were approached until

at about the 6 mm ramifications they constituted the principal type of cell. In all the bronchi the smooth muscle layer was conspicuous. The glands of the submucosa in both the trachea and the bronchi were actively secreting.

Almost all of the bronchi whose internal diameters were less than 5 mm, together with the bronchioles and even the alveolar ducts, were filled with Curschmann's spirals. These consisted of twisted layers of fibrin, mucus, detritus, nuclear fragments, many eosinophils and fewer plasma cells, lymphocytes and recognizable sloughed epithelial cells. The underlying walls of these portions of the bronchial tree disclosed two distinct but intimately intermingled pathologic processes. The first was a diffuse infiltration of the type seen in bronchial asthma, and the second was an acute granulomatous lesion approaching the type seen in the fulminating "soft tubercle" type of tuberculosis. The former consisted of extension and accentuation of the lesion already described in connection with the trachea and larger bronchi (fig 2). Gradually the epithelial cells showed more and more replacement with large droplets of mucus until in the bronchioles only a few scattered cells were still recognizable as epithelial cells and even these were distorted by an abundant amount of mucoid material. Cilia were not seen. Everywhere the basement membrane was broadened, hyalinized and conspicuous. The rest of the wall was greatly thickened and composed of a background of edematous fibrous and connective tissue wherein the muscle fibers and glands were partially or completely destroyed. Capillaries were engorged and numerous. There was a diffuse and dense infiltration of eosinophils and fewer plasma cells and lymphocytes. Whereas ordinarily this diffuse inflammation was confined to the bronchial and bronchiolar walls, it sometimes extended into the immediately adjoining alveolar septums.

Throughout the walls of about two thirds of the smaller bronchi and bronchioles and extending from the basement membrane to the cartilages and even beyond there was a second, granulomatous type of lesion (figs 2 and 3). This consisted of many small and fairly uniform-appearing foci composed of a central area of complete necrosis and a surrounding zone of perpendicularly arranged epithelioid cells. While most of the latter were of the ordinary mononuclear variety, a few were grouped together to form rather spurious giant cells consisting of two to four eccentrically or peripherally placed nuclei and an abundance of pink cytoplasm. Immediately surrounding and partly infiltrating the zone of epithelioid cells there were a few plasma cells and lymphocytes, and these were in turn surrounded by varied numbers of eosinophils. The latter blended with the adjoining inflammatory reaction already described. Although most of the tubercles were of the necrotic variety, a few were more solid and in place of the central areas of necrosis disclosed collections of round or oval mononuclear or multinuclear giant cells intermingled with definite epithelioid cells. In most of the smaller bronchi these granulomatous foci were confined to the bronchial walls, but in the bronchioles they were in direct communication with conglomerate foci of an exactly similar nature that infiltrated both the adjoining alveolar walls and the alveolar spaces (fig 4).

Numerous sections of the apical scar of the left lung disclosed a mass of hyalinized acellular fibrous tissue, which contained scattered deposits of carbon pigment. Along one border this hyalinized mass was covered with rather cellular fibrous tissue, the edges of which contained numerous active tubercles of the type already described (fig 5). These infiltrated the alveolar sep-

tums and spaces and, as in other portions of the lungs, were sometimes seen surrounding bronchioles. Although serial sections were not made, several sections from each block did not disclose any areas of old necrosis or calcification within the fibrous tissue. Sections of other portions of each lung disclosed chronic emphysema alternating with areas of atelectasis. None of the vessels showed recent, old or healed periarteritis nodosa. Only those lymph nodes at the hilus of the left lung were available for histologic study, and none of them showed any evidence of recent or old tuberculosis.

Microscopic sections of the nasal mucosa disclosed a covering of nonciliated, nonsecreting transitional epithelium several cell layers thick. The basement membrane was thick and hyalinized. The glands were numerous and actively secreting and were supported by dense fibrous tissue, which was infiltrated with lymphocytes, plasma cells and scattered eosinophils. Tubercles were not observed. In each maxillary sinus the lining membrane was about ten times the normal thickness. It was mostly denuded of its epithelium, having only a single or a double basilar layer of nonciliated cells (fig 6). As in the nose, the basement membrane was hyalinized and conspicuous. The submucosa showed marked edema and was heavily infiltrated by eosinophils and fewer plasma cells and lymphocytes. The former were particularly abundant immediately beneath the basement membrane. In this region there were also numerous tubercles of exactly the same structure as those already described in the lungs.

Giemsa-stained sections of the lungs rendered particularly conspicuous the eosinophils and the necrotic foci of the tubercles but failed to disclose any bacteria, fungi or inclusion bodies in any of the lesions. Acid-fast stains of duplicate sections disclosed many acid-fast granules but no tubercle bacilli. Elastic tissue stains with Van Gieson's counterstain showed complete disruption of both the elastic tissue and the muscle bundles in areas involved with the granulomatous process.

Routine sections of the heart, all the abdominal organs and the marrow of the lumbar vertebrae failed to show either tubercles or periarteritis nodosa.

#### COMMENT

That the patient whose case is described in this report had true bronchial asthma is borne out clinically by (1) the sudden appearance of typical attacks of asthma four years previously which until the last attack were readily and completely controlled with epinephrine and (2) the fact that she was known to be sensitive to some of the commoner allergens, although the exact nature of these was not disclosed in the recorded history. The clinical impression of bronchial asthma is further supported by the rather typical pathologic changes observed in the lungs. Grossly, they were voluminous, light, teathy and dry and the bronchi and bronchioles were filled to capacity with mucous plugs. Microscopically, there were blocking of the lumens of the smaller bronchi and bronchioles with Curschmann's spirals, conversion of the epithelial cells into goblet cells, which were distended with mucus and showed a loss of their cilia, hyalinization and conspicuous widening of the basement membrane, great thickening of the submucosa by edematous connective and fibrous tissue and

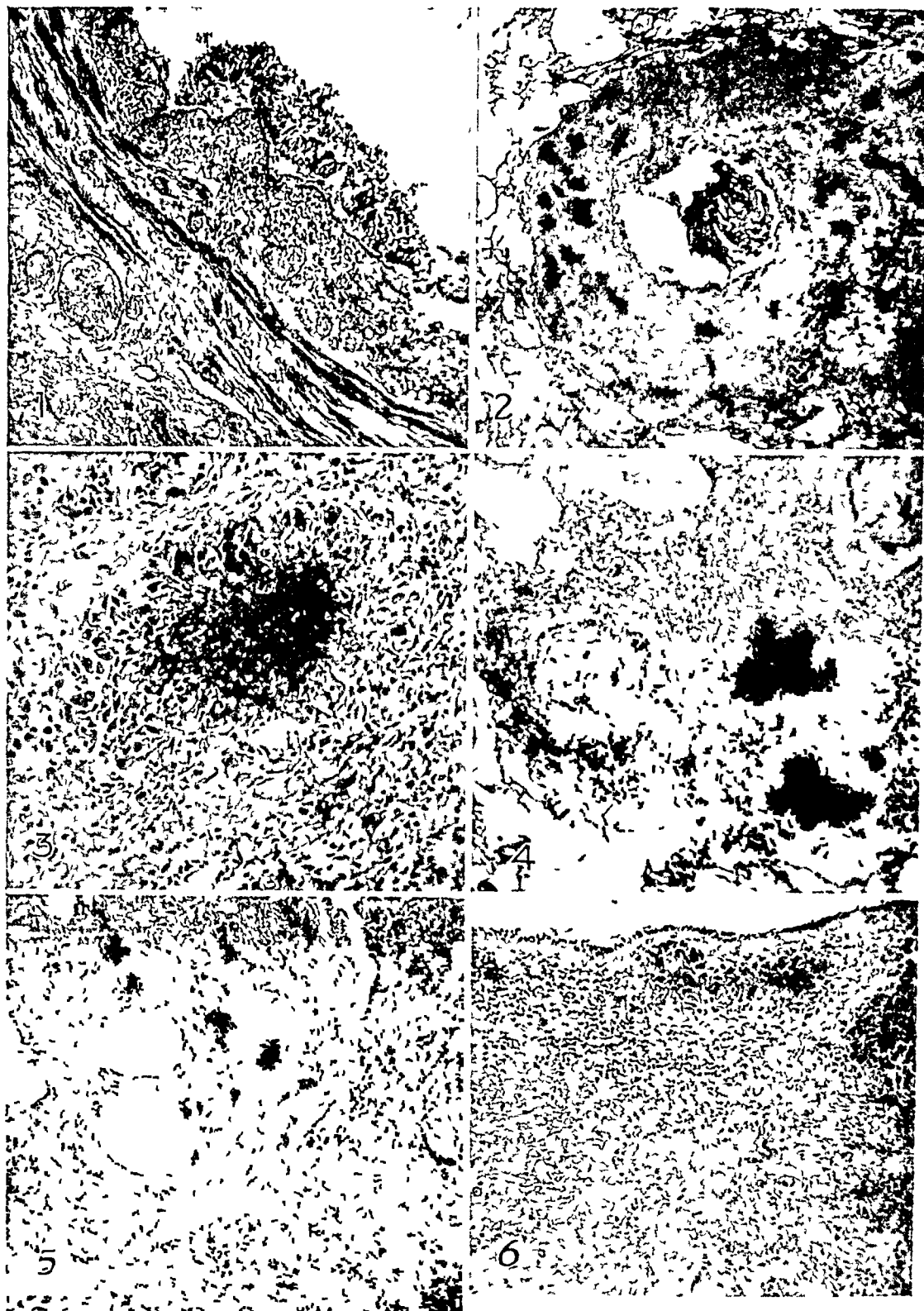


Fig 1—Section of a large bronchus in which the lumen contains debris, the wall is partially denuded of mucosa, the remaining epithelial cells are without cilia, the basement membrane is prominent and hyalinized, the submucosa is edematous and congested, with a cellular infiltration in which eosinophils are abundant, and the muscle bundles are prominent. Hematoxylin and eosin,  $\times 75$ .

Fig 2—A small bronchus partially occluded with mucus and detritus. The epithelium that remains contains vacuoles. The entire wall is greatly thickened by asthmatic granulation tissue in which eosinophils predominate and by numerous tubercles. Hematoxylin and eosin,  $\times 25$ .

Fig 3—High magnification of bronchiolar tubercle. Central area of necrosis is surrounded by epithelioid and giant cells. At the periphery are lymphocytes, plasma cells and farther out many eosinophils. Giemsa stain,  $\times 200$ .

Fig 4—Peribronchiolar tuberculous pneumonia with extension of this process to the walls of the bronchioles, which are already involved with a lesion typical of asthma. Hematoxylin and eosin,  $\times 37.5$ .

Fig 5—Section from scar of apex of left lung showing from below up dense scar tissue containing carbon pigment, more cellular fibrous tissue and tubercles along "spreading border." Hematoxylin and eosin,  $\times 37.5$ .

Fig 6—Section of the mucosa of the right antrum showing partial epithelial denudation with loss of the cilia of the remaining epithelial cells, prominence and hyalinization of the basement membrane, congestion and edema of the submucosa and diffuse infiltration by eosinophils. To the right, immediately beneath the basement membrane, there are two small tubercles and a portion of a larger one. Hematoxylin and eosin,  $\times 75$ .

diffusely infiltrating eosinophils, hypertrophy and hypersecretion of the submucosal glands and hypertrophy of the muscle bundles of the bronchi. These changes in the bronchial mucosa have often been described in the past,<sup>4</sup> and while they are considered as characteristic of bronchial asthma by some authors they are considered as non-specific by others.<sup>5</sup> Rackemann<sup>6</sup> has recently stated that if one differentiates between true asthma and asthma-like conditions, particularly in patients in whom asthma develops after the age of 40, one will find that the microscopic changes in the bronchial tree are highly characteristic. To this view I fully subscribe and therefore consider the histologic alterations described in this report as specific for the disease.

What applies to the mucosa of the tracheo-bronchial tree applies equally to the mucosa of the nasal and paranasal sinuses of asthmatic patients. Unfortunately, permission to examine the head and so the sphenoid, ethmoid and frontal sinuses was not granted, but it is reasonable to assume that the changes in these membranes were similar to those seen in the mucosa of the maxillary sinuses. It is rather surprising that, although histologic studies of the mucosa of the nasal and paranasal sinuses<sup>7</sup> and of the trachea, bronchi and bronchioles have been made independently on many occasions, both parts of the respiratory tract have seldom been studied in the same patient.<sup>8</sup> In the case reported here it is clearly seen that the lesion of the upper portion of the respiratory tree is so similar to that of the lower portion that one could almost be superimposed on the other.

The diagnosis of tuberculosis in this case was based entirely on the histologic appearance of the granulomas. The peripheral collections of epithelioid and giant cells surrounding either central foci of eosinophilic necrosis or more densely arranged epithelioid and giant cells appeared to be midway between the "soft" and the "hard" tubercles described by Rich.<sup>9</sup> The one disturbing factor was the failure to demonstrate any definite tubercle bacilli which Rich

maintained is easy to do in the "soft" tubercles. This failure may be explained by the fact that even the most recent tubercles were not as acute as the "soft" tubercles referred to, as evidenced by the always present zone of epithelioid cells and scattered giant cells. In such lesions one would expect fewer tubercle bacilli and so more difficulty in demonstrating them. Acid-fast granules, however, were present in most of the necrotic centers. Because the lesion was not suspected at the time of necropsy, cultures for tubercle bacilli and inoculations of guinea pigs were not carried out. Since "soft" tubercles, which the lesions here approach, are found only in hypersensitive persons, the disease as described was a result of a reinfection, and the primary tuberculous focus was represented by the apical scar in the upper lobe of the left lung. Although a central area of caseation, such as those which are sometimes seen to persist in such scars, was not noted, the spreading edge of this fibrous nodule<sup>9</sup> was quite characteristic of a relighted tuberculous lesion.

The exact mechanism by which the tuberculous process was disseminated throughout the respiratory system is difficult to ascertain with any degree of certainty. There are three observations, however, that point to an air-borne spread. First, the infection was limited to the respiratory passages alone and was not found in the intervening parenchyma of the lung. If the dissemination had been blood borne, one would expect to find it throughout all portions of the lungs if a pulmonary artery was eroded, or throughout the entire body if the bacilli gained entrance into a pulmonary vein. Second, the distribution of the tubercles was typical of that seen with an air-borne spread, namely, the presence of small foci of tuberculous pneumonia beyond and about the bronchioles and alveolar ducts with a subsequent secondary involvement of the walls of these terminal portions of the bronchial tree.<sup>9</sup> Third, however common pulmonary tuberculosis is, it is unusual to find tuberculosis of the paranasal sinuses.<sup>10</sup> The presence of tubercles in the superficial portions of the mucous membranes of each antrum, that is, just beneath the basement membrane, bespeaks air-borne infection, for had it been blood borne the lesions would not only have been in the deeper portions of the submucosa but, as already stated, in the other organs of the body as well. Although the source of the aerogenous reinfection was not absolutely divulged, it was probably a reanimated focus in the upper lobe of the left lung. Because, however, an area of old necrosis was not seen in that "healed" lesion, the following two alternate sources may be mentioned: (1) another more active focus in the

4 (a) Huber, H. L., and Koessler, K. K. *Arch Int Med* **30** 689, 1922. (b) Kountz, W. B., and Alexander, H. L. *Arch Path* **5** 1003, 1928. (c) Thieme, E. T., and Sheldon, J. M. *J Allergy* **9** 246, 1938. (d) Michael, P. P., and Rowe, A. H. *ibid* **6** 150, 1934. (e) MacDonald, I. G. *Ann Int Med* **6** 253, 1932. (f) Hilding, A. C. *Ann Otol, Rhin & Laryng* **52** 5, 1943.

5 (a) Tuft, L. *Clinical Allergy*, Philadelphia, W. B. Saunders Company, 1937, p. 327. (b) Lamson, R. W., Butt, E. M., and Stickler, M. *J Allergy* **14** 396, 1943.

6 Rackemann, F. M. *Arch Int Med* **73** 248, 1944. Lamson and others.<sup>5b</sup>

7 Semenov, H. *J A M A* **111** 2189, 1938. Grove, R. C. *New York State J Med* **41** 455, 1941.

8 Kountz and Alexander.<sup>4b</sup> Michael and Rowe.<sup>4d</sup>

9 Rich, A. R. *The Pathogenesis of Tuberculosis*, Springfield, Ill., Charles C. Thomas, Publisher, 1944, pp. 819, 856 and 852.

10 Hersh, J. H. *Arch Otolaryng* **28** 987, 1938. Radner, D. B., and Pinkerton, F. J. *Am Rev Tuberc* **50** 313, 1944.

right lung which was not found but which had discharged its contents into a bronchus and (2) an entirely new exogenous infection

Since the patient had both asthma and tuberculosis, the final question to be answered is the extent to which the tuberculosis contributed to the asthmatic attacks. From a consideration of both the clinical and the pathologic data it is apparent (1) that the asthma was the initial and older lesion and that the tuberculosis was only more recently superimposed, (2) that because the two lesions were so intimately intermingled the diameters of the terminal portions of the bronchial tree were narrowed not only by the asthmatic granulation tissue but by the tubercles as well, and therefore in this manner each contributed to the obstruction and the dyspnea, and (3) that in the areas of tuberculous involvement the muscle bundles in the walls of the bronchi and bronchioles were completely disrupted by the tubercles. Although it is known that in status asthmaticus epinephrine<sup>24</sup> may have little or no beneficial effect, the lack of response to this drug in the case reported here may have been related to the destruction

of the muscle fibers. While muscle spasm in the larger bronchi, where the bundles were intact, undoubtedly contributed to the severity of the asthmatic attacks and was probably relieved by the epinephrine, such relief was not possible in the more peripheral portions where the muscles were destroyed and the occlusion was due entirely to mucous plugs and thickening of the wall by cellular infiltration.

#### SUMMARY

That bronchial asthma and tuberculosis can occur concomitantly in the same patient is well established, but the morphologic relationship of the two diseases is not apparent from a review of the literature. In the case presented a patient 50 years old who died in status asthmaticus and in whom asthma had developed four years previously was found on histologic examination to have in the mucosa of the upper and lower respiratory tree an intimate intermingling of asthmatic granulation tissue with tuberculous lesions. It is concluded therefore, that the latter can contribute appreciably to the occlusion of the air passages and so to the asthmatic attacks.



# General Reviews

## NEOPLASTIC DISEASES OF THE HUMAN HYPOPHYSIS

JOHN E KRAUS, M D

PEORIA, ILL

### THE ADENOMA

The enormous amount of literature on this topic proves the frequency of tumor formation in the hypophysis. In 1914 Kraus,<sup>1</sup> after examining 300 hypophyses in serial sections, stated that 25 glands were involved by adenoma, while 13 showed adenomatous hyperplasia. Among 1 000 unselected persons Castello<sup>2</sup> encountered adenoma of the hypophysis in about 1 of every 4 cases. The greatest incidence of adenoma occurred in persons in the sixth decade of life, males and females were involved about equally. Among 50 cases of primary tumor of the hypophysis Puestow<sup>3</sup> found adenoma in 60 per cent, in 15 per cent of which it was carcinomatous. According to Kraus,<sup>1</sup> adenoma involves the hypophysis in 30 per cent of persons from 50 to 60 years of age, in 15 per cent of persons from 30 to 50 and only in 6 per cent of persons below 20 years of age. Ecker<sup>4</sup> investigated the frequency of small basophilic adenoma and found it in 54 of 721 hypophyses. The frequency of chromophobe, chromophil and mixed adenoma was examined by Bailey,<sup>5</sup> who observed that the chromophobe type was three times more frequent than the two chromophil types. The incidence of adenoma and craniopharyngioma as compared with other intracranial blastomas becomes evident from Bailey's statistical work based on 2,000 cases. According to Bailey hypophysial adenoma represents 17.8 per cent and craniopharyngioma 4.3 per cent of all intracranial tumors. A similar result was obtained by Henderson,<sup>6</sup> who observed hypophysial adenoma in 17 per cent of all persons with tumor of the brain.

The anatomic picture of hypophysial adenoma varies greatly. While in some instances it is tiny and thus macroscopically hardly discernible

in others there is present a huge tumor mass protruding from the enormously enlarged sella turcica and destroying the neighboring parts of the brain and the cranial base by pressure or infiltration or both. As long as it does not exceed a certain size, its growth takes place predominantly in the lateral direction and downward toward the bottom of the sella, since the diaphragm of the sella withstands the abnormal pressure longer than the osseous wall of the sella. With the adenoma growing larger, the entrance to the sella gets wider, and the tumor projects through the diaphragm into the cranial space. Thus an extrasellar portion of the tumor arises which sometimes considerably exceeds in size the intrasellar portion. The two portions, the intrasellar and the extrasellar, in typical cases are differentiated by a circular ring of constriction giving an hourglass shape to the tumor. The larger growth may invade one or both cavernous sinuses, extend to the carotid vessels, destroy the sella and break into the sphenoid and ethmoid sinuses and even into the nasopharynx and the maxillary sinuses (Cushing<sup>7</sup>, Bailey and Cutler<sup>8</sup>, Spark and Biller<sup>9</sup>). A tumor extending posteriorly destroys the dorsum sellae and the clivus, a tumor may penetrate the dura, grow outside the dura along the blood vessels and reenter the subdural space, forming nodules on the inner surface of the dura. Growing toward the foramen opticum, the adenoma may invade the orbits.

Growing toward the brain, the adenoma comes into contact with this organ below, behind or in front of the optic chiasm. This is pushed upward and becomes flattened, together with the optic tracts and the optic nerves. The optic nerves become atrophic, sometimes constricted and even severed as a result of pressure against the medial cerebral artery or other branches of the circle of Willis. In rare instances the chiasm is perforated by the tumor. In its further growth, the adenoma burrows more and more deeply into the diencephalon, causing atrophy and destruction of the floor of the third ventricle. With

From the Department of Pathology, St Francis Hospital

1 Kraus E J Beitr z path Anat u z allg Path **58** 159, 1914

2 Castello, R T Am J Path **12** 205, 1936

3 Puestow, C B Arch Neurol & Psychiat **22** 547, 1929

4 Ecker, A D Endocrinology **23** 609, 1938

5 Bailey, P Tumors of the Hypophysis Cerebri, in Penfield, W Cytology and Cellular Pathology of the Nervous System, New York, Paul B Hoeber, Inc., 1932, vol 3, p 1131

6 Henderson, W R Brit J Surg **26** 811, 1939

7 Cushing, H Intracranial Tumors, Springfield, Ill, Charles C Thomas, Publisher, 1932

8 Bailey, O T, and Cutler, E C Arch Path **29** 368, 1940

9 Spark, C, and Biller, S Arch Path **35** 93, 1943



a larger tumor the peduncles, occasionally the temporal lobes and rarely the frontal lobes are pushed apart and compressed by portions of the tumor. Sometimes the tumor breaks through the floor of the third ventricle and projects into the greatly dilated ventricle, pushing apart the thalami optici and corpora striata and crowding out the fornix and the corpus callosum. The tumor may not only displace these structures but infiltrates them. The question to what extent expansive, and to what extent infiltrative, growth has participated in the destruction of the adjacent parts is sometimes macroscopically hard to determine. Experience shows that even with cancerous adenoma the destruction is due more to the pressure exerted by the tumor than to infiltration.

Besides the optic nerves, other nerves adjacent to the tumor, such as the oculomotor and trochlear nerves, less frequently the abducent or trigeminal nerves, are crowded out, stretched and flattened. Thus paralysis of ocular nerves is not rare, as is shown by Weinberger, Adler and Grant,<sup>10</sup> who, among 160 cases of adenoma of the hypophysis, noted 14 in which paralysis of ocular nerves dominated the clinical picture (cavernous sinus syndrome).

The injury done by the adenoma to the remainder of the hypophysis varies in severity. Even in some cases of adenoma of large size the hypophysis, lying at the base of the deepened sella, may be in a fairly good condition, whereas in other cases the hypophysis is destroyed or reduced to a flat, atrophic structure. Concentrically arranged layers of atrophic cells forming a thin shell frequently are found surrounding the basal parts of the tumor. In case of predominantly extrasellar growth, the hypophysial stalk becomes destroyed at an early stage of the advance. In the vast majority of cases adenoma arises from the anterior lobe. Basophilic adenoma springs from both the anterior and the posterior lobe. Occasionally the origin of adenoma can be traced to foci of anterior lobe parenchyma dispersed along the craniopharyngeal canal within the sphenoid bone. If the adenoma is eosinophilic in nature, acromegaly may result.<sup>11</sup> In rare instances adenoma starting from the pharyngeal hypophysis has been recorded. It is represented by a retropharyngeal mass between the pharynx and the spine.<sup>12</sup>

10 Weinberger, L. J., Adler, F. H., and Grant, F. C. *Arch Ophth* **24** 1197, 1940.

11 Erdheim, J. *Beitr z path Anat u z allg Path* **46** 233, 1909.

12 Leegard. *Norsk mag f lægevidensk* **78** 829, 1917.

Though adenoma of the hypophysis frequently is carcinomatous, metastases are seldom observed. Multiple metastatic nodules in the leptomeninges, even as far as the cauda equina, have been seen. Extension of a carcinomatous adenoma up to the occipital foramen, with the tumor breaking into the pons, the medulla and the bulbous venae jugularis, with metastases to the lungs and the pleura, has also been observed. An eosinophilic adenoma with metastases to the skeleton has been described by Vasilu<sup>13</sup>, a carcinoma with abdominal metastases by Gilmour.<sup>14</sup>

There are as many types of hypophysial adenoma as there are cell types in the hypophysis. The histogenesis of these tumors is expressed by placing before "adenoma" the name of the cell type from which the adenoma has originated. Thus one speaks about chief cell adenoma, fetal cell adenoma, pregnancy cell adenoma, eosinophilic adenoma and basophilic adenoma. When more than one cell type is represented in the adenoma, the tumor is called mixed cell adenoma. Mixed cell adenoma is composed of (1) chief cells and transitional cells, (2) these cells together with chromophilic cells, (3) transitional and chromophilic cells and (4) fetal cells and chief cells. The term "malignant adenoma" is used oftener than the term "carcinoma" in order to emphasize the difference which exists between these tumors and ordinary carcinoma. It should be borne in mind that the cells in carcinomatous adenoma are not always differentiated enough enough to reveal their true histogenesis. For instance, in the cases of carcinomatous adenoma without demonstrable specific cell granules, the term "carcinomatous chromophobic adenoma" might be used. It also should be mentioned that the tumor cells in old eosinophilic adenoma may lose their specific granules to such an extent that unless examined by specific methods thoroughly, the eosinophilic character of the adenoma may be overlooked. This might have happened in some cases of "acromegaly without eosinophilic adenoma."

While eosinophilic and chromophobic forms of adenoma seem to arise almost exclusively in the anterior lobe, basophilic adenoma is found, as said, in the anterior as well as in the posterior lobe—in the former, however, much more frequently than in the latter. In Cushing's disease it has been seen in either lobe. Crooke's "hyalin change" of the basophilic cells, supposedly characteristic of Cushing's disease, was noted by Ecker<sup>4</sup> in 55 per cent of cases of basophilic

13 Vasilu, T. *Virchows Arch f path Anat* **276** 141, 1930.

14 Gilmour, M. D. *J Path & Bact* **35** 265, 1932.

adenoma without Cushing's disease, whereas in the rest of the cases the hypophysis was free of any such change. Ecker, of course, in these cases missed ballooning of the nuclei, excessive vacuolation, tendency to multinucleation and general enlargement of cells. It may be mentioned here that basophilic adenoma, as a rule, does not attain the size of chromophobic or eosinophilic adenoma.

There is one type of chromophobic adenoma in which the cells are similar in shape and arrangement to those in the anterior lobe during the very early stage of fetal life. For this reason, Kraus,<sup>1</sup> who described the tumor first in 1914, called it fetal or fetal cell adenoma, since he assumed that it originated from remnants of these fetal cells, which are sometimes found in the anterior lobe. These tumors are characterized by high cylindric or fusiform cells arranged in bandlike interlacing strands. Thus arise cell palisades which, resting on the septums and the capillary walls, confer on the new growth a characteristic appearance (Kraus<sup>15</sup>, Berblinger<sup>16</sup>, Shapiro<sup>17</sup>). Circumscribed hyperplasia, as well as circumscribed nodules made up of these cells, are not rare. Usually the nodules are small, however, in a case reported by Kraus the tumor was the size of a hazelnut and infiltrated the hypophysial capsule as well as the adjacent parenchyma. In carcinomatous types, diffuse proliferation of the tumor parenchyma without particular cell arrangement may be seen in some parts while the characteristic picture described is seen in other parts.

Another rare type is the so-called pregnancy cell adenoma which, as the name indicates, is made up of cells morphologically corresponding to the normal pregnancy cells. These little nodules represent more a form of adenomatous hyperplasia than real adenoma. The structure of the hypophysial adenoma varies, with the cells taking an alveolar or a perivascular or a diffuse arrangement, adenoma with papillary and in rare instances cylindromatous structures<sup>18</sup> also may be seen.

The adenoma in the hypophysis sometimes seems to spring from a local cell hyperplasia showing a well defined contour only on one side whereas on the other side the cells proliferate diffusely, merging into the adjacent hypophysial parenchyma. To outline the new

growth from the neighboring cells in places where a distinct margin is missing, fat stains with osmium or sudan are used to differentiate the newly formed tissue from the normal parenchyma. The new growth shows a lack of lipoids as compared with the considerable lipoid content of the normal tissue. Such a nodule, partly without sharp outlines, is called adenomatous hyperplasia. It is not rare, especially in the posterior lobe, where it is made up of basophilic cells, evidently springing from the basophilic cells normally present in the posterior lobe.

The question as to whether the chromophilic adenoma originates from chief cells or exclusively from chromophilic cells has been discussed often. The fact that in some cases chief cells are present together with chromophilic cells, linked by transitional cells, seems to indicate that in at least some cases chromophilic adenoma springs from chief cells which by further differentiation turn into chromophilic elements. It is allowable, however, to assume that chromophilic adenoma also arises directly from chromophilic cells as, for instance, in the posterior lobe, where basophilic adenoma may be seen emerging from the basophilic cells regularly found in this part of the organ.

In some instances it might be difficult to distinguish microscopically between simple and cancerous adenoma. When infiltrative growth can be proved, there cannot be any doubt of carcinoma. The presence of granules in the cells, although these cells may show high differentiation, is no proof of simple adenoma. The most reliable sign of carcinoma aside from infiltrating growth is anaplasia of the tumor cells, characterized by irregularity in size and shape of cells and nuclei and by particularly large size of the latter, which exceed the size of nuclei in adenoma. While alveolar arrangement of the tumor cells is usually noted in adenoma, a more diffuse arrangement suggests carcinoma. In instances of cancerous adenoma, the formation of cell strands is maintained, but these are broader than those in adenoma. The frequently observed perivascular arrangement of the tumor parenchyma is also suggestive of carcinoma. This perivascular arrangement has caused, especially in older publications, misinterpretation of adenoma as, for example, perithelioma, perivascular sarcoma or angiosarcoma.

Infiltrative growth of basophilic adenoma in the posterior lobe is not always a reliable criterion of carcinoma. This adenoma springs from the basophilic cells, which physiologically invaded the posterior lobe by infiltration. They may maintain this behavior, which of course does not prove that the growth is carcinoma, at least

<sup>15</sup> Kraus, E. J. The Hypophysis, in Henke, F., and Lubarsch, O. *Handbuch der pathologischen Anatomie und Histologie*, Berlin, Julius Springer, 1926, vol. 8, p. 810.

<sup>16</sup> Berblinger, W. *Handbuch der inneren Sekretion*, Leipzig, Curt Kabitzsch, 1932, vol. 1, p. 910.

<sup>17</sup> Shapiro, P. F. *Arch. Path.* **11** 22, 1931.

<sup>18</sup> Jedlička, V. *Sborník* **25** 149, 1924.

as long as it remains confined to the posterior lobe, only after it has invaded other structures, can carcinoma be diagnosed. In some instances adenoma turns into carcinoma—an incident that as far as the eosinophilic adenoma is concerned, according to Spark and Biller,<sup>9</sup> occurs in about 20 per cent of the cases.

Retiographic lesions in cases of hypophysial adenoma are not rare. They are chiefly necrosis, hyaline degeneration of the interstitial tissue and formation of hyaline concentric-layered bodies with or without calcification. An adenoma with calcareous concretions is called struma calcuosa or adenoma psammosum. Calcification may also occur in the form of amorphous, irregularly shaped calcium deposits. Several times petrified adenoma has been described as stone-hard in consistency and coarsely granulated on the cut surface (Kraus<sup>15</sup>). The microscopic picture may show numerous round psammous bodies, as those in the choroid plexus, closely approximated, with little tumor parenchyma left between them. Psammous adenoma can be suggested from the roentgenogram, which discloses a shadow within the tumor (Deery<sup>19</sup>). The same features, of course, are seen more often in old forms of craniopharyngioma which undergo calcification.

Among the circulatory lesions in adenoma, hemorrhages are most common. In rare instances the whole adenoma may undergo hemorrhagic infarction, apparently due to thrombosis or perhaps compression of veins. An old hemorrhage may become encapsulated and form a hemorrhagic cyst. Trauma may be the inciting cause of a hemorrhage in the tumor, as has been shown by Van Wagenen<sup>20</sup>. Edema (together with slight fibrosis) was seen by Goldberg and Lisser<sup>21</sup> in an eosinophilic adenoma after it had been irradiated with roentgen rays.

To determine the site of origin of adenoma is possible only when the tumor is small. The chief cell adenoma and the adenoma made up of transitional cells prefer the peripheral parts of the anterior lobe, the small pregnancy cell adenoma affects the lateral parts, occurring especially in the corner between the border of the posterior lobe and the lateral portion of the capsule of the anterior lobe. The eosinophilic adenoma lies more in the central part and the basophilic adenoma in the central part but close to the posterior lobe or in the posterior lobe itself. The fetal cell adenoma is found in both

the central and the peripheral parts of the anterior lobe.

Hypophyses with adenoma sometimes show small so-called hemias, gaps up to 1 mm in width, in the capsule of the anterior lobe. These contain prolapsed parenchyma, may be single or multiple and arise apparently where vessels pass through the capsule. Increased pressure within the hypophysis may cause these small ruptures.

#### THE CRANIOPHARYNGIOMA

The tumor second in frequency to the adenoma is the craniopharyngioma which is derived from the squamous epithelium of the former craniopharyngeal canal. In many cases small groups of these cells are found in the anterior and superior parts of the anterior lobe of the hypophysis as well as in the pars infundibularis. When the craniopharyngioma springs from cell groups dispersed into the anterior lobe, an intrasellar craniopharyngioma develops, when it arises from cell groups in the pars infundibularis, the result is a suprasellar craniopharyngioma. While craniopharyngioma in this location, especially above the sella, is not rare, only a few cases are known in which the tumor developed within the sphenoid bone, in the region of the former craniopharyngeal canal proper (Zeitlin<sup>22</sup>). Why just the intrasphenoid craniopharyngioma appears to be so rare can be explained only by the fact that the epithelial remnants of the craniopharyngeal canal do not persist within the bone with the same frequency as in the aforementioned parts of the hypophysis.

There are simple and carcinomatous forms of craniopharyngioma, partially solid, partially cystic or cystic-papillary in structure. In many cases both solid and cystic parts are seen. These tumors vary greatly in size, attaining in rare cases the size of a man's fist. The intrasellar craniopharyngioma crowds out the hypophysis, causing extreme atrophy and widens and deepens the sella. Growing larger, it projects out of the sella and displaces the neighboring diencephalon upward. It may destroy the sella and even larger parts of the sphenoid bone as far as the pharyngeal wall, pushing the latter downward. Growing toward the optic chiasm, it usually invades the brain in front of the chiasm. Nodular portions of the craniopharyngioma can break into the lateral ventricle and into the frontal lobes. If the craniopharyngioma is cancerous, it infiltrates the adjacent parts of the skull and the brain and sometimes sets up metastases to the brain. Occasionally a can-

19 Deery, E. M. *Endocrinology* **13** 455, 1929.

20 Van Wagenen, W. P. *Ann Surg* **95** 625, 1932.

21 Goldberg, M. B., and Lisser, H. *J Clin Endocrinol* **2** 477, 1942.

22 Zeitlin, H. *Am J Cancer* **23** 729, 1935.

cerous craniopharyngioma breaks through into the pharynx after it has penetrated the cranial base along the blood vessels and nerves. Metastatic nodules have been found in the ependyma of the fourth ventricle and may simulate large ependymal granulations. Metastases to the cervical lymph nodes also have been described.

Different pictures result when the craniopharyngioma springs from the infundibular part of the hypophysis (outside the sella turcica). It then lies within the circle of Willis and behind the optic chiasm and is attached above to the base of the brain and below to the hypophysis. The floor of the third ventricle is pushed upward and is often extremely thinned or even destroyed, with the result that a large part of the tumor lodges within the ventricle. Sometimes the hypophysis is about normal, but more often it is compressed. The circle of Willis may be lifted and stretched by the tumor, the optic nerves may be indented by one of the arteries and pressed between the growing tumor and the big arteries or they may even be severed.

Histologically, craniopharyngioma is characterized by typical prickle cells, which lack keratohyaline granules as well as cornification. The solid parts of the tumor are made up of squamous cell nests closely approximated and embedded in a fibrous stroma, only the periphery of these cell nests is built by a single layer of cylindric epithelium. Frequently there are regressive changes in both parenchyma and stroma, particularly hydropic degeneration, which by liquefaction often causes pseudocysts. It is believed that the liquefaction is due to poor nutrition of the tissue from hyaline degeneration of the blood vessels.

If the whole cell nest is liquefied, the necrotic mass, stained red by eosin, lies amid the stroma, often surrounded by foreign body giant cells, or else it undergoes calcification. The craniopharyngioma may contain bone tissue which has developed from these calcified foci by metaplasia.<sup>23</sup> Deposits of hemosiderin and cholesterol crystals also are found in specimens of this tumor. The presence of bone and brown pigment, the latter interpreted as melanin, has led to confusing craniopharyngioma with teratoma.

In cystic and cystic papillary craniopharyngioma the lining of the cysts is made up of two or more layers of epithelium, occasionally flattened by the cyst content. The lining shows a basal cell layer and layers of prickle cells, but without keratinization. Characteristic are shaggy vegetations budding from the wall, being attached

to it either by a broad base or by thin stalks forming cauliflower-like structures. The papillae are covered by multilayered squamous epithelium and sometimes have an edematous stroma rich in blood vessels. Destruction of the epithelial lining by necrosis, presence of foreign body giant cells, deposition of calcium and cholesterol and ossification are found in the cystic type as well as in the solid type. The cystic content may be colorless, yellow brownish or brown-green, it may be serous, mucous or gelatinous in nature and sometimes shows a large amount of glittering cholesterol crystals. Characteristic of craniopharyngioma is its resemblance to adamantinoma, which has caused many authors to call craniopharyngioma adamantinoma. Finally it is quite typical of craniopharyngioma that the carcinomatous type occurs more often in young persons, whereas the simple form usually is found in older ones. The two sexes are about equally involved.

#### OTHER PRIMARY TUMORS

Little fibromas have been seen in the hypophyseal stalk as well as in the hypophysis proper. Jedlička<sup>18</sup> has described a fibroma the size of a pea in the pars intermedia together with a partially patent craniopharyngeal canal in a 14 year old child with macrosomia. Hemangioma of the anterior lobe and lipoma of the posterior lobe are rare. A hypophyseal chordoma was observed by Goerke.<sup>24</sup> A few instances of sarcoma of the hypophysis have been described such as the instance of fibrosarcoma reported by Willis.<sup>25</sup> Many cases of sarcoma (angiosarcoma, round cell sarcoma, perithelioma and others) described in the old literature were misinterpreted instances of chromophobic adenoma. Glioma and ganglioneuroma of the posterior lobe are mentioned in the literature several times. Globus<sup>26</sup> gave the name "infundibuloma" to a tumor of the hypophyseal stalk duplicating the histologic appearance of the neurohypophysis. Globus observed the tumor in children.

Teratoma also belongs to the rare tumors of the hypophysis or hypophyseal region. The hypophysis may be found compressed and atrophic beside the tumor. The craniopharyngeal canal may be patent, as in a case reported by Gautier,<sup>27</sup> in which the teratoma developed close to the cranial opening of the canal, penetrated the anterior lobe and grew into the third ventricle. In the cases examined, colloid-filled

<sup>23</sup> Critchley, M., and Ironside, R. N. *Brian* **49** 437, 1926

<sup>24</sup> Goerke, M. *Folia oto-laryng* **20** 9, 1930

<sup>25</sup> Willis, R. A. *M. J. Australia* **1** 287, 1930

<sup>26</sup> Globus, J. H. *J. Neuropath. & Exper. Neurol* **1** 59, 1942

<sup>27</sup> Gautier. *Frankfurt Ztschr. f. Path.* **19** 247, 1916

cysts, teeth, bone cysts lined with ciliated epithelium, fat tissue, blood vessels, glia, ganglion cells and other structures have been observed. In other cases anlage material of almost all organs was found. In fetuses and newborn infants complicated teratoma containing fetal parts has been observed in connection with epignathus. Usually there is an intracranial portion in the sellar region in connection with a tumor mass protruding from the mouth, both parts being linked by a narrow middle part running through a defect in the sphenoid bone. Thus the whole tumor assumes the characteristic shape of an hourglass (Baart de la Faille<sup>28</sup>, Kraus<sup>29</sup>). A specimen described by the latter showed a teratoma composed mainly of anlage material of the brain and the eye with islands of cartilage and other mesodermal derivatives. Berblinger<sup>16</sup> in his monograph cited a case reported by du Marchie Sarvaas<sup>30</sup> in which in a boy 12 years of age a huge teratoma had destroyed the hypophysis and the sella and penetrated into the nasal cavity. The tumor contained bone, cartilage, various gland formations (among them mucous glands), glia and other tissues.

Cholesteatoma has been described several times, though it seems not to occur in the hypophysis itself but in the infundibular region. It is of epidermal origin as proved by the presence of keratohyaline granules which are not found in craniopharyngioma. In a case recorded by Kraus<sup>31</sup> there was a cholesteatoma (the size of a hazelnut) lodged in the angle between the hypophyseal stalk and the upper surface of the anterior lobe, the latter not being involved by the tumor. In other cases a cholesteatoma developing at the base of the brain may take the form of a large tumor obliterating the hypophysis<sup>32</sup>.

True cysts of the hypophysis are not frequent. The macroscopic distinction from cystic craniopharyngioma may be difficult to determine in some cases. For instance, a cyst derived from the pars intermedia grossly may simulate cystic craniopharyngioma. Remnants of anterior lobe tissue lining the cyst facilitate the histologic diagnosis. In most of the cases the cyst of the pars intermedia is intrasellar and separates the two lobes. It can be lined by ciliated and also

squamous epithelium (Fulstow<sup>33</sup>, Jedlička<sup>18</sup>). A tumor in the region of the sella turcica which was lined with ciliated epithelium was described by Frazier and Alpers<sup>34</sup> and interpreted as a true tumor of Rathke's cleft. In some instances the cyst is not followed by clinical symptoms, in some, however, it has been observed to cause Simmonds' disease, testicular atrophy (Jedlička<sup>18</sup>) and other pathologic changes. Whether or not there are clinical symptoms depends on the size of the cyst and the degree of compression and atrophy of the hypophysis itself. The size of the cyst varies from case to case, in the majority it is not large. The size in a case described by Jedlička 5 by 3.8 by 3.4 cm. must be regarded as a rare size.

Another type of cyst is the ependymal cyst, usually located within the infundibulum and lined by ependyma. Rarely it becomes larger in size than in a case described by Jedlička in which it developed in the infundibulum, passed through the hypophyseal stalk and the diaphragm of the sella into the sella and caused marked pressure atrophy of the hypophysis without, however, causing any endocrine symptoms.

#### METASTATIC TUMORS

Metastatic carcinoma is found more frequently in the hypophysis than metastatic sarcoma. The most frequently observed metastases are derived from carcinoma of the breast, the lung, the bronchial tree and the thyroid gland, but records show that carcinoma of the stomach, the prostate, the kidney and other organs also metastasizes to the hypophysis. According to Wohlwill,<sup>35</sup> who thoroughly examined 177 persons with carcinoma, hypophyseal metastases are rare, they occurred in only 5 persons. Metastatic carcinoma in the majority of cases first involves the posterior lobe, from which it often extends to the adjacent parts of the anterior lobe. In some instances the entire hypophysis is destroyed by cancer. The anterior lobe is the primary site of the metastasis less frequently than the posterior lobe. There can be a diffuse carcinomatous infiltration of the anterior lobe while the posterior lobe contains several small nodules. Nodular metastases of carcinoma in the anterior lobe are rare. Carcinoma of the prostate usually invades the sella first and then progresses into the hypophysis. Carcinoma of the nasopharynx or of the sphenoid sinus invading the hypophysis was observed by me in a few instances. When

28 Baart de la Faille, cited by Kraus<sup>15</sup>.

29 Kraus, E. J. Virchows Arch f. path. Anat. **271** 546, 1929, in Schwalbe, E. Morphologie der Missbildungen des Menschen und der Tiere, edited by G. B. Gruber, Jena, Gustav Fischer, 1929, vol. 3, p. 32.

30 du Marchie Sarvaas, G. J. Frankfurt Ztschr. f. Path. **40** 210, 1930.

31 Kraus, E. J. Med. Klin. **20** 1290 and 1328, 1924.

32 Krumbhaar, E. B. M. Clin. North America **5** 927, 1921. Kraus<sup>15</sup>.

33 Fulstow, M. Am. J. Path. **4** 87, 1928.

34 Frazier, C. H., and Alpers, J. B. Arch. Neurol. & Psychiat. **32** 973, 1934.

35 Wohlwill, F. Deutsche Ztschr. f. Nervenheil. **105** 62, 1928.

mainly involving the posterior lobe, metastatic carcinoma may be a cause of diabetes insipidus. Carcinoma of the bronchi, the breast, the thyroid gland and the stomach are the most common types to metastasize thus. Wohlwill<sup>35</sup> pointed to the interesting fact that in many cases of advanced carcinoma (in 45 among his 177 cases) the patient shows an unexpected well developed panniculus adiposus. This can be explained partly by changes in the endocrine system and the vegetative centers in the diencephalon. In 27 of Wohlwill's cases it was his opinion that the cachexia associated with tumor was prevented by the atrophy of the ovaries and the destruction of the hypophysis and the floor of the third ventricle by metastatic tumor.

Besides metastatic carcinoma, metastatic lymphosarcoma, mesothelioma of the pleura and melanoblastoma are mentioned in the literature several times. Cell emboli of melanoblastoma caught in capillaries of the anterior lobe have been observed by me. In a case of primary

melanosarcomatosis of the leptomeninges I noted infiltration of the hypophysial stalk. Metastatic sarcoma usually invades the hypophysis after it has destroyed the sphenoid bone. Hematogenous metastasis of sarcoma as recently reported by Kraus<sup>36</sup> in a case of primary sarcoma of the ueter occurs less often than extension of sarcoma, as from the base of the skull or from the pharynx (Jedlička<sup>18</sup>) or from the dura or the brain, to the hypophysis. Such extension may cause diabetes insipidus (Beiblinger)<sup>16</sup> or adiposogenital dystrophy. Worms and Delater,<sup>37</sup> however, reported a case in which there was complete destruction of the hypophysis by a nasopharyngeal lymphosarcoma without any symptoms of hypophysial insufficiency. It is also known that diabetes insipidus resulting from a metastasis in the posterior lobe may disappear after the tumor has invaded and destroyed the anterior lobe.

---

36 Kraus, E. J. *Urol & Cutan Rev* **48** 522, 1944

37 Worms and Delater. *Rev neurol* **32** 361, 1925

## Book Reviews

**Bronchial Asthma** By Leon Unger, B S, M D, assistant professor in the department of medicine, Northwestern University Medical School, Chicago. Pp 724, with 123 illustrations. Price \$9. Springfield, Ill. Charles C Thomas, Publisher, 1945.

It is now about twenty-five years since the intensive study of bronchial asthma as an allergic disease began. This book by a pioneer clinician in this special field presents the advances in the understanding, the prevention and the treatment of asthma during that time. The first and longest section deals with the clinical side of allergic asthma—the causes, the pathologic appearances, the symptoms, the diagnosis, the prevention and the treatment. There are chapters also on other allergic diseases and on the military aspects of asthma and other allergies. The historical chapter with its interesting portraits is noteworthy. The second section describes the preparation of the various allergenic extracts used in testing and treatment. The appendix contains a mass of useful information about the sources of allergens, house dust, diets, instructions for patients and other topics. Each chapter is provided with a concise summary and a list of references. In several cases the references cover many pages. The charts and the pho-

tographs serve their purpose nicely. Any one interested in allergic asthma will find this book a rich source of information.

**Trauma in Internal Diseases, with Consideration of Experimental Pathology and Medicolegal Aspects** Rudolf A Stern, M D, assistant attending physician, City Hospital, New York. Pp 575. Price \$6.75. New York. Grune & Stratton, 1945.

In 1899 the first edition of Rudolf Stern's "Traumatische Entstehung innerer Krankheiten" was published by Gustav Fischer. The last major revision of the German edition was made in 1930 by Richard Stern, son of Rudolf. Although much of the present book has been taken almost verbatim from the German text, it is more than a translation. A considerable amount of new material, particularly from American authors, has been added. The most serious fault of the book arises from the fact that the author, like his predecessors, tends to designate as probable certain cause and effect relationships that would be better characterized as possible. In spite of this, the book is to be regarded as a welcome addition to the clinical literature dealing with the etiologic importance of mechanical injuries in the production of disease.

## News and Comment

**Appointments and Retirements**—James W Jobling, Delafield professor of pathology and head of the department of pathology in the College of Physicians and Surgeons, Columbia University, New York, will retire from routine and administrative work on July 1 next.

Helen Ingleby has resigned from the professorship of pathology in the Woman's Medical College of Pennsylvania and will be pathologist of the Jewish Hospital in Philadelphia.

Andrew C Ivy, professor of physiology at the Northwestern University Medical School, has been appointed a member of the National Advisory Cancer Council of the United States Public Health Service.

Alvin M Pappenheimer, professor of pathology in the College of Physicians and Surgeons, Columbia University, New York, will retire July 1 next.

Jeff Minckler, formerly member of the department of pathology in the St Louis University School of Medicine, has been named assistant professor of pathology in the University of Oregon Medical School.

Harry Pratt Smith, professor and head of the department of pathology in the University of Iowa, has been appointed professor and executive officer of the department of pathology in the College of Physicians and Surgeons, Columbia University, New York.

**Death**—Hans Sachs, formerly professor of immunology at the University of Heidelberg, Germany, who was connected with Trinity College in Dublin, Ireland, where he had a fellowship, died on March 28, 1945.

**Awards**—Edwin J Cohn, professor of biochemistry at Harvard Medical School, is the first recipient of the Passano Foundation Award of \$5,000. This foundation was established in 1944 to aid in research which bears promise of clinical application. The award was given Dr Cohn in recognition of his work on blood plasma.

At a recent meeting of the American Foundation for Tropical Medicine the Richard Strong Medal for outstanding service in the field of tropical medicine was awarded to Rear Admiral Edward R Stutt, former surgeon general of the United States Navy.

**Fellowships**—The Permanente Foundation Hospital Oakland, Calif, offers fellowships for clinical research, paying \$225 a month plus maintenance. Requests for detailed information should be addressed to the fellowship committee of the hospital.

The Jessie Horton Koessler Fellowship of the Institute of Medicine of Chicago for the aid of research in biochemistry, physiology, bacteriology or pathology will be available on September 1. The stipend is \$500 a year with the possibility of renewal for one or two years. Applications must be approved by the head of a department in the fields mentioned or by the director of a research institute or laboratory in Chicago, and it is stipulated that the recipient of the fellowship shall be given adequate facilities for carrying out the proposed research. Applications by letter with full information about the research should be sent to Dr Paul R Cannon, 950 East Fifty-Ninth Street, Chicago 37, before July 1 next.



## POIKILOCYTOSIS IN DAIRY CATTLE

J T REID, MS, C F HUFFMAN, PH D

AND

C W DUNCAN, MS

EAST LANSING, MICH

The term "poikilocytosis" was first used by Quincke<sup>1</sup> in 1880 to designate a pathologic condition of the blood in which variously shaped deformed erythrocytes called poikilocytes appeared. The poikilocytic condition is characterized by various bizarre-shaped erythrocytes which vary greatly in size and shape. The degree of distortion may be extensive or slight, depending on the nature and the severity of the blood disease. Many shapes of poikilocytes have been observed which do not fall into any named class characterized by their particular form. However, various forms and shapes have been described: tailed,<sup>2</sup> filamented,<sup>3</sup> club-shaped,<sup>2c</sup> dumbbell,<sup>4</sup> sickle,<sup>5</sup> spherical,<sup>6</sup> anvil,<sup>4</sup> tennis

racquet,<sup>7</sup> stellate,<sup>8</sup> tomahawk or hatchet,<sup>9</sup> gourd,<sup>7a</sup> teardrop,<sup>2b</sup> kidney,<sup>9</sup> cigar,<sup>4</sup> sausage shape,<sup>2b</sup> horseshoe,<sup>7a</sup> oat shape,<sup>2b</sup> dagger,<sup>7a</sup> pencil,<sup>4</sup> target,<sup>10</sup> dimpled,<sup>6a</sup> and boot shaped.<sup>2b</sup>

There are no reports in the literature which indicate that poikilocytosis is a primary disease in itself, and no uniform explanation accounts for the formation or the presence of the poikilocytes in the blood. A number of theories have been proposed, however: (a) Arneth (cited by Kanellis<sup>1b</sup> and Haden<sup>11</sup>) expressed the belief that poikilocytes are produced in the bone marrow, (b) Isaacs,<sup>12</sup> Cunningham,<sup>4</sup> Dameshek<sup>10b</sup> and Sydenstricker<sup>13</sup> were in agreement on the hypothesis that the cells are "congenitally deformed," (c) according to Osler,<sup>3d</sup> they are produced by an altered condition of the serum, whereas (d) Penati<sup>14</sup> and Emmel<sup>3a</sup> favored the belief that erythrocytes are transformed to poikilocytes in the circulating blood.

Bohrod<sup>15</sup> indicated, however, that there is an accentuation of the eccentricity of the elliptic cell after its removal from the body which explains the formation of this type of malshape-ness after exposure to the atmosphere. Whether or not this theory explains the production of poikilocytes, it necessitates caution in handling the blood specimen. Other investigators<sup>16</sup> have also suggested the necessity of careful technic in the manipulation of the blood sample in order

From the Departments of Dairy and Agricultural Chemistry and the Institute of Nutrition, Michigan State College.

Published with the approval of the director of the Michigan Agricultural Experiment Station as Journal Article 739 (ns).

1 (a) Quincke, H I, cited by Osler,<sup>3d</sup> vol 3, p 882, (b) Kanellis, E S *Folia haemat* **35** 65, 1927

2 (a) Kracke, R R *Diseases of the Blood and Atlas of Hematology*, ed 4, Philadelphia, J B Lippincott Company, 1941, p 95 (b) Osgood, E E, and Ashworth, C M *Atlas of Hematology*, San Francisco, J W Stacey, Inc, 1937, p 90 (c) Kernkamp, H C *H Technical Bulletin* 87, Minnesota Agricultural Experiment Station, 1932

3 (a) Emmel, V E *Arch Int Med* **20** 586, 1917 (b) Takeuchi, K *Folia haemat* **34** 259, 1927 (c) Rossle, R *ibid* **34** 281, 1927 (d) Osler, W *Diseases of the Blood and Blood Glandular System. A System of Practical Medicine*, Philadelphia, Lea Brothers & Co, 1885

4 Cunningham, R S, in Nelson's *New Loose-Leaf Medicine*, New York, Thos Nelson & Sons, 1941, vol 4, p 3

5 (a) Cooley, T B, and Lee, P *Am J Dis Child* **32** 334, 1926 (b) Huck, J G *Bull Johns Hopkins Hosp* **34** 335, 1923 (c) Herrick, J B *Arch Int Med* **6** 517, 1910 (d) Sydenstricker, V P, Mulherin, W A, and Houseal, R W *Am J Dis Child* **26** 132, 1923 (e) Cheney, G J *A M A* **98** 878, 1932 (f) Kracke<sup>2a</sup> Emmel<sup>3a</sup>

6 (a) Haden, R L, and Evans, F D *Arch Int Med* **60** 133, 1937 (b) Ponder, E *The Mammalian Red Cell and the Properties of Haemolytic Systems*, Berlin, Verlagsbuchhandlung Gebruder Borntraeger, 1934, p 75

7 (a) Da Costa, J C *Clinical Hematology*, ed 2, Philadelphia, P Blakiston's Son & Co, 1905, p 183 (b) Osgood and Ashworth<sup>2b</sup> (c) Cunningham<sup>4</sup>

8 Whitlock, S C *J Wildlife Man* **3** 14, 1939

9 Cunningham<sup>4</sup> Da Costa<sup>7a</sup>

10 (a) Bohrod, M G *Am J M Sc* **202** 869, 1941 (b) Dameshek, W *ibid* **200** 445, 1940

11 Haden, R L *Am J M Sc* **188** 441, 1934

12 Isaacs, R *Handbook of Hematology*, New York, Paul B Hoeber, Inc, 1937, vol 1, p 5

13 Sydenstricker, V P *J A M A* **83** 12, 1924

14 Penati, F *Arch per le sc med* **54** 189, 1930

15 Bohrod, M G *Elliptical Erythrocytes Relation to Crenation*, unpublished data, 1941

16 (a) Wintrobe, M M *Clinical Hematology*, Philadelphia, Lea & Febiger, 1942, pp 51, 67 and 81 (b) Osgood and Ashworth<sup>2b</sup> (c) Cunningham<sup>4</sup> (d) Da Costa<sup>7a</sup>

to avoid confusing the poikilocytes with artefacts Da Costa <sup>7a</sup> stated that poikilocytosis is akin to crenation so far as the cells in both conditions may be similarly distorted and misshapen. It is unlike crenation, however, for the reason that poikilocytosis is a pathologic condition, demonstrable the moment the blood is withdrawn from the body, whereas crenation is a physical phenomenon dependent on external influences for its production. Crenation never occurs until the blood has remained exposed to the air for some time. Osgood and Ashworth <sup>2b</sup> regarded crenation as an artefact produced by faulty preparation of the blood specimen and stated that the crenated forms may be differentiated from the poikilocytes by the regularly notched outline and nodular surface which are characteristic of all of the cells in a particular area. Kanellis, <sup>1b</sup> Cunningham, <sup>4</sup> and Wintrobe <sup>16a</sup> called attention to the possible confusion of poikilocytes with crenated erythrocytes produced artificially by trauma during the preparation of the blood film. Other physical influences which distort the shape of the red blood cell are (a) diluting the blood sample in saline solution, <sup>17</sup> (b) drying blood films slowly, <sup>2b</sup> (c) heat, <sup>18</sup> (d) hemorrhage <sup>3b</sup> and (e) irradiation with roentgen rays <sup>1b</sup>. Different chemical substances have been shown to produce changes in normal erythrocytes resulting in shapes which resemble poikilocytes. Sodium chloride, <sup>19</sup> alkali dissolved from glass, <sup>20</sup> mercuric chloride, <sup>3c</sup> lecithin and cholesterol, <sup>1b</sup> and alanine <sup>21</sup> are some of the substances which will alter the shape of the red cells.

Research on poikilocytes occurring alone in animals is quite limited. Its occurrence in connection with human diseases has been studied in a secondary manner. Little is known concerning its cause and mechanism. However, the appearance of poikilocytes has been observed and described as observed in different species of animals and in human beings not only in disease but also in health. Many workers <sup>22</sup> observed

poikilocytes with many bizarre shapes in blood from patients with pernicious anemia. Takeuchi <sup>3b</sup> found filamented red blood cells and detached filaments in 69 per cent of observed cases of tuberculosis, carcinoma, intestinal disorder and fever, in addition to those seen in cases of pernicious anemia. Kilduffe <sup>22a</sup> pointed out that abnormally shaped erythrocytes may appear in any case of severe or long-standing anemia. Elliptic red corpuscles, according to Floiman and Wintrobe, <sup>23</sup> are noted in most cases of anemia, and sometimes more than 25 per cent of the cells are affected. Da Costa <sup>7a</sup> reported the general occurrence of small, slightly deformed poikilocytes in milder types of anemia and larger, extremely distorted poikilocytes in the more severe types. Penati <sup>14</sup> associated elliptically deformed erythrocytes with pernicious anemia, secondary anemia and hemolytic icterus, but he found no relationship between the intensity of cell deformation and the severity of the anemia, although the deformed cells became less noticeable with amelioration of the anemia.

Sickle cell anemia (first named by Mason <sup>24</sup> in 1922 but first described by Herrick <sup>25</sup>) is a condition in which a specific and constant type of poikilocytosis is associated with anemia. Huck, <sup>5b</sup> Sydenstricker <sup>13</sup> and Alden <sup>25</sup> observed the sickle cells in the blood of male and female patients characterized by anemia and a tendency to have ulcers on the legs. Sydenstricker <sup>13</sup> reported sickle cell anemia occurring with abdominal pains and pathologic changes of the spleen. Cells of peculiar sickle shapes were noted in tissue sections and blood films by Landon and Lyman <sup>26</sup> in 1929. According to Emmel, <sup>3a</sup> the sickle cell trait appears to be hereditary and occurs most commonly in the Negro race although the reports of Hunter and Adams, <sup>27</sup> Lawrence <sup>28</sup> and Cooley and Lee <sup>29</sup> showed the occurrence of this condition in other races. Van den Bergh <sup>29</sup> and Huck <sup>5b</sup> demonstrated that this condition may be inherited by either sex as a dominant trait.

Dameshek <sup>10b</sup> reported a type of anemia characterized by target cells, which constituted as much as 32 per cent of the erythrocytes, and

17 (a) Apinis, P. *Acta Univ. latv., Vet. Med. Fak.* **5** 443, 1941; *Vet. Bull.* **11** 194, 1941. (b) Gough, A. *Biochem. J.* **18** 202, 1924.

18 (a) Levy, J. *Arch. Path.* **7** 820, 1929. (b) Hahn, E. V., and Gillespie, E. B. *Arch. Int. Med.* **39** 233, 1927. (c) Ponder, E. *The Erythrocyte and the Action of Simple Haemolysins*, Edinburgh, Oliver & Boyd, 1924. (d) Koeppe, H. *Arch. f. d. ges. Physiol.* **99** 33, 1903. (e) Kanellis <sup>1b</sup>. (f) Takeuchi <sup>3b</sup>.

19 Rossle <sup>3c</sup>. Apinis <sup>17a</sup>.

20 Waller, W. W. *J. Physiol.* **56** 218, 1922.

21 Nicolaeff, N. M. *Folia haemat.* **42** 116, 1930.

22 (a) Kilduffe, R. A. *The Clinical Interpretation of Blood Examinations*, Philadelphia, Lea & Febiger, 1931, p. 200. (b) Quincke <sup>1a</sup>. (c) Kanellis <sup>1b</sup>. (d) Takeuchi <sup>3b</sup>. (e) Cunningham <sup>4</sup>. (f) Da Costa <sup>7a</sup>. (g) Isaacs <sup>12</sup>.

23 Florman, A. L., and Wintrobe, M. M. *Bull. Johns Hopkins Hosp.* **63** 209, 1938.

24 O'Roke, E. C. *Proc. Soc. Exper. Biol. & Med.* **34** 738, 1936.

25 Alden, H. S. *Am. J. M. Sc.* **173** 167, 1927.

26 Landon, J. T., and Lyman, A. V. *Am. J. M. Sc.* **178** 223, 1929.

27 Hunter, W. C., and Adams, R. B. *Ann. Int. Med.* **2** 1162, 1929.

28 Lawrence, J. S. *J. Clin. Investigation* **5** 31, 1927.

29 Van den Bergh, A. A. *Arch. f. Verdauungskr.* **43** 65, 1928.

resembling somewhat Cooley's sickle cell anemia. He designated this condition "target-cell anemia" although this type of cell was found to be associated with other types of anemia. Bohrod<sup>10a</sup> stated that the target cells appear for a short time in acute types of anemia but disappear as the erythrocyte count rises. In chronic types of anemia the target cells exist for a long period in quantities as great as 10 per cent of the erythrocytes. Haden and Evans<sup>61</sup> observed target cells in several different types of anemia but found them in great numbers in sickle cell anemia. Wintrobe<sup>16a</sup> associated various undifferentiated poikilocytes and target cells with Mediterranean anemia. Isaacs<sup>12</sup> in studies of blood films of patients having primary lesions of the bone marrow observed abnormal shapes in some of the red blood cells. The presence of poikilocytes in myelogenous leukemia and chlorosis was indicated by Emmel.<sup>31</sup>

Mrowka<sup>30</sup> reported the presence of red corpuscles of spindle or elliptic shape in the blood of horses suffering from infectious anemia. Poisonous substances elaborated by many helminths were regarded by Hutyrá and Marek<sup>31</sup> as a cause of poikilocytosis and anemia. Bernhard<sup>32</sup> reported what he termed "oval poikilocytosis," which was accompanied by anemia and a splenic tumor. Apinis<sup>33</sup> observed filamented red blood cells in the blood of horses, cattle, sheep, dogs, cats, rabbits, pigs and chickens. The filaments varied for different animals in a distinctive manner. Thin filaments were noted on the erythrocytes of hogs, very long filaments on those of chickens and button-like structures on those of sheep and cats, and granulation was noticeable in the red blood cells of the horse. He gave no description of the erythrocytes observed in the blood of cattle. O'Roke<sup>24</sup> and Whitlock<sup>8</sup> observed sickle cells, stellate cells, rod-shaped cells and other poikilocytes in the blood of deer. Hanke and Koessler<sup>34</sup> reported poikilocytes in the blood of guinea pigs showing symptoms of scurvy. The observation of spindle-shaped poikilocytes in fowl's blood was made by Gordon.<sup>35</sup>

Vawter<sup>36</sup> made microscopic examinations of the blood of dairy cattle and found poikilocytosis

associated with anaplasmosis. He concluded that this is one of several clinical findings which distinguish anaplasmosis from bacillary hemoglobinuria (or red water disease) in dairy cattle. Blount<sup>37</sup> noted a few sickle cells in the blood of a day old calf which he thought were artefacts. He observed no poikilocytes in the normal adult bovine animal. Neal and Ahmann<sup>38</sup> reported that anisocytosis and poikilocytosis were observed in their studies of anemia in dairy calves of Florida. Knoop and others<sup>39</sup> showed a photomicrograph of a blood smear from a milked calf with severe anemia which demonstrated extensive poikilocytosis.

From a review of the literature it is obvious that only limited studies have been made of poikilocytosis in bovine animals. The object of this paper is to report the occurrence and the symptoms of poikilocytosis and the association of the condition with health and disease of dairy animals receiving various farm rations.

#### PROCEDURE

Calves of different ages representing Holstein, Guernsey, Jersey and Ayrshire breeds, cows representing Holstein, Guernsey and Jersey breeds, 5 cows with fistula of the rumen in the herd of the Michigan State College and dairy animals in herds on farms of Michigan were used in this investigation. The major portion of the data, however, was derived from calves in the experimental herd of the Michigan State College.

The animals in most cases were bled weekly and often several times during the week. Jugular blood was used for microscopic examination, hemoglobin determinations and other tests employed in the investigation. All blood samples except those used for microscopic examination were collected in test tubes containing lithium citrate as the anticoagulant. Since several authors have indicated that contamination with certain reagents and faulty manipulation in preparing a specimen for examination may result in artefacts which resemble the poikilocyte, all known precautions were employed to avoid these errors. Only freshly shed blood was used since the common anticoagulants are salts capable of increasing the osmotic pressure sufficiently to effect possible alterations of shape. Immediately after the blood was drawn, a small drop was placed on a clean slide, spread over its surface with a clean coverslip and dried as rapidly as possible.

Some hanging drop preparations were made also as a check on the blood smears, with an isotonic solution of sodium chloride or blood plasma being used as the suspension medium. A few drops of blood were drawn through a paraffin-lined rubber tube attached to a paraffin-lined bleeding needle which was submerged below the surface of the suspension medium contained in a receptacle. This apparatus prevents direct contact of the blood with the atmosphere. The blood was mixed in the medium and the hanging drop was prepared and sealed in a depression slide for microscopic examination.

30 Mrowka, F. *Vet Bull* 6 174, 1935

31 Hutyrá, F., and Marek, J. *Special Pathology and Therapeutics of the Diseases of Domestic Animals*, Chicago, Alex. Eger, 1926, vol 2, p 339

32 Bernhard, G. *Klin Wchnschr* 6 1493, 1927

33 Apinis, P. *Wien Tierarztl Monatschr* 23 680, 1938

34 Hanke, M. T. and Koessler, K. K. *J Biol Chem* 80 499, 1928

35 Gordon, L. *Virchows Arch f path Anat* 262 1938, 1926

36 Vawter, L. R. *Norden News* 17 4, 1943

37 Blount, W. P. *Vet J* 95 222, 1939

38 Neal, W. M., and Ahmann, C. F. *J Dairy Sc* 30 741, 1937

39 Knoop, C. E., Krauss, W. E., and Washburn, R. G. *J Dairy Sc* 18 337, 1935

Weekly hemoglobin determinations were made by the method of Sanford, Sheard and Osterberg<sup>40</sup> Standard procedures were employed for the blood cell count and the determination of cell volume

RESULTS

*Incidence of Poikilocytosis*—During the course of this investigation the blood from 423

TABLE 1—*Distribution of Given Degrees of Poikilocytosis in Affected Animals*

Percentage of Poikilocytes, Range	Affected Animals	
	Number	Percentage
1 10	81	34.7
11 20	31	13.2
21 30	21	9.0
31 40	13	5.5
41 50	19	8.1
51 60	15	6.8
61 70	9	3.8
71 80	12	5.1
81 90	18	7.8
91 100	14	6.0
Total	233	100.0

animals was studied, 190 of which showed no evidence of poikilocytosis. The number and the percentage of animals affected with poikilocytes and the percentage of poikilocytes in the blood are shown in table 1. In 78 animals the poikilocytes constituted more than 51 per cent

TABLE 2—*Incidence of Poikilocytosis in Three Different Herds in One Community*

Animal	Percentage of Poikilocytes	Hemoglobin Content, Gm per 100 Cc	Red Blood Cell Count
Unaffected Herd			
R1	0	13.7	• 7,840,000
R2	0	12.5	
R3	0	11.2	
R4	0	12.5	
R5	0	10.1	
R6	0	11.7	6,940,000
R7	0	12.8	
R8	0	11.3	
R9	0	10.9	
R10	0	10.5	
Affected Herds			
N1	78	10.5	8,380,000
N2	95	11.4	8,160,000
N3	95	11.6	
N4	50	11.6	
T1	50	14.3	7,480,000
T2	65	13.3	
T3	95	10.5	10,100,000

of the total red blood cells, in 53 animals, more than 61 per cent, in 44, more than 71 per cent, in 32, more than 81 per cent, and in 14, more than 91 per cent.

It was observed that one or more herds in a community would tend to show a high incidence

<sup>40</sup> Sanford, A. H., Sheard, C., and Osterberg, A. E. *Am J Clin Path* 3:405, 1933.

of poikilocytosis while the neighboring herd or herds would be entirely free from this condition. Table 2 shows data on two herds with poikilocytosis and an unaffected herd in the same community.

*Biconcave Disk-Shaped Erythrocytes*—The microscopic observations of the blood smears from the 423 animals reveal that 190 had normal biconcave disk-shaped erythrocytes. Symmetric, regularly outlined round corpuscles with transparent-appearing centers suggesting biconcavity were noted. By the use of the hanging drop preparation, in which the erythrocytes could be observed from many different angles, it was found that the predominant shape of the erythrocytes in normal, healthy dairy cattle is the biconcave disk. Figure 1 shows a photomicrograph of a blood smear of a representative animal showing the normally shaped red corpuscles.

*Poikilocytes*—Almost all of the shapes of the poikilocytic cells described in the literature were observed in this study. In addition, many other bizarre forms were seen to which no descriptive name has been applied. The most prevalent poikilocyte encountered consists of a "body" of variable shape and size from which tail-like structures protrude. The tail-like protrusions varied from 2 to 7 microns in length. The number of tails per cell commonly varied from one to five and were arranged in a haphazard fashion around the body of the cell. Frequently, cells possessing a round "body" were observed to have one or two of the protruding structures. The tail-like structures were a definite part of the cell and were stained homochromically with the rest of the cell. The severity of the poikilocytic condition seemed to be associated with the length of the tail-like protrusions. Figure 2 is a photomicrograph of a blood smear showing many shapes of poikilocytes in the blood of a representative animal.

Many other shapes suggestive of the following objects were seen: urn, half moon, star, oval cell, sickle, tear drop, pipe, horseshoe, kidney, tennis racquet, Christmas tree, gondola, tomahawk, dagger, oat, sausage and gourd. All of them often appeared with tail-like or filamented structures identical with those already described. The degree of distortion of cell and irregularity of shape was more accentuated in the bloods that contained the larger numbers of poikilocytes. The extreme contrasting appearance of normal erythrocytes and severely poikilocytic cells is best illustrated by an examination of figures 1 and 2.

*Cells with Crenation Induced by Hypertonic Saline Solution*—It was observed early in this

investigation that the possibility of confusing poikilocytes with crenated corpuscles was slight. Crenation induced in normally shaped corpuscles with 2 per cent saline solution is manifested by a somewhat symmetric and regular arrangement

phenomenon was seen on the surface of the corpuscle as well as at the periphery. A comparison of the poikilocytes in figure 2 with the crenated forms in figure 3 evidences this observation. The crenated shapes in figure 3 are those

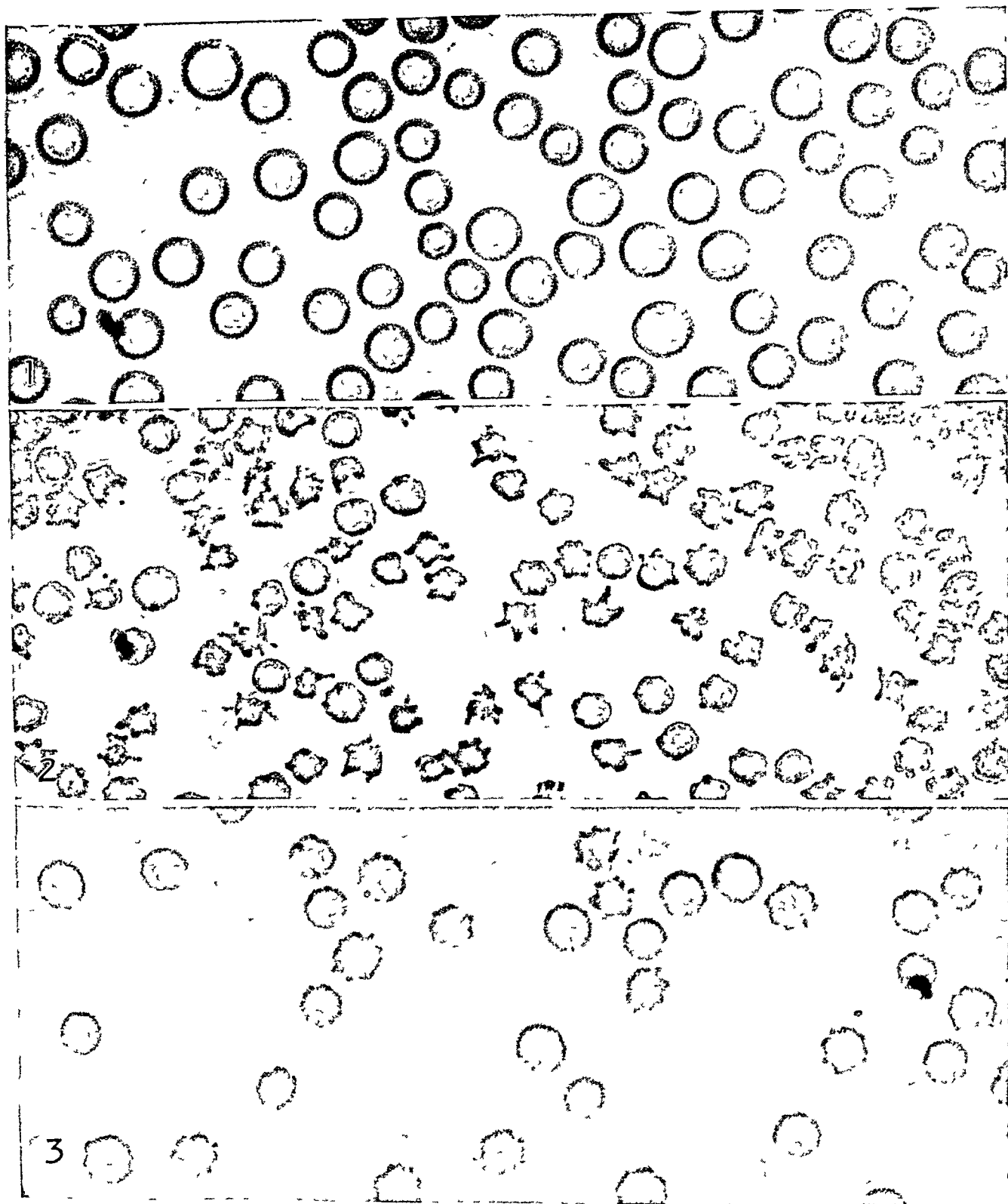


Fig 1—Photomicrograph of a blood smear showing normal bovine biconcave disk-shaped erythrocytes,  $\times 1,270$

Fig 2—Photomicrograph of a blood smear showing poikilocytic cells,  $\times 1,270$

Fig 3—Photomicrograph of a blood smear showing crenated cells produced from the same normal corpuscles shown in figure 1,  $\times 1,270$

of nodular or short spinelike structures around the periphery of the corpuscle when viewed in a blood smear. In hanging drop preparations this

induced in the normal corpuscles shown in figure 1 with 2 per cent saline solution. The regular arrangement of nodules and other features of

the crenated forms offer a sharp contrast to the different bizarre forms exhibited by the poikilocytes in figure 2

Several stages of crenation were observed which were apparently due to the severity of the process. In the early stage the corpuscle displays slight wrinkling and bulging of the surface and the periphery. Later in the process the wrinkled and bulged foci of the cell become more intensified in the form of knobs or spinelike nodules. These structures appear to develop into longer, protruding coarse filaments, which are arranged in a regular fashion around the cell, and later finer, fuzzy, hairlike processes develop in place of the coarser protrusions.

*Health of Animals Affected with Poikilocytosis*—The health of the animals whose blood showed 50 per cent or more of the total red corpuscles to be poikilocytes was usually subnormal. Anorexia was the outstanding symptom that accompanied high incidence of poikilocytosis. In some instances, however, depraved appetite was manifested by the animal's chewing of wood. Other symptoms shown by the affected animals were general thinness and roughness. The hair was long and shaggy and had a hard, dry appearance. Noticeable features shown by the young animals were a marked retardation of growth and a general unthrifty condition.

The poikilocytic status of the affected animals was not influenced by the plasma concentration of calcium, inorganic phosphorus or magnesium or by the hemoglobin content or the number of or the volume of red cells, since the values obtained did not reveal any constant feature that could be associated with the degree of severity of the poikilocytosis.

#### COMMENT

In order to make a study of poikilocytosis in dairy cattle it was necessary to establish a standard for the normal shape of the erythrocyte, especially since the reports in the literature are controversial and quite limited with regard to bovine animals. The red corpuscles of animals which were apparently normal in all respects showed a circular biconcave disklike shape when observed in hanging drop or blood smear preparations. The hanging drop preparation in which the corpuscles were suspended in either isotonic solution of sodium chloride or blood plasma was particularly adapted to the study of corpuscular shape "in vitro" since it permitted the observation of the corpuscle from many different viewpoints. The stained red cells in blood smears showed a round, regular outline and a heavily stained rim at the periphery of the cell

with a light or nonstaining inner portion, suggesting a biconcave disk shape as the normal erythrocyte shape. No investigation has definitely established the normal in vivo shape of the bovine erythrocyte, and the studies of the normal shape in vitro are also recorded in a somewhat contradictory manner. The form observed in this study, however, is in close agreement with the bovine corpuscular shape described by Blount<sup>37</sup>

Many investigators have shown that normally shaped corpuscles manifest alterations in conformation as a result of having been contaminated by different physical and chemical agents during the preparation of the blood specimen. Since the occurrence of such artefacts would result in confusion in the study of cell shape, every precaution was taken to avoid those agents which are capable of effecting changes in the shape of the cells. Crenated corpuscles were reported<sup>41</sup> to be the artefacts most commonly encountered and those most nearly resembling the poikilocytes. It was found during this investigation that the chances of confusing the crenated forms with the poikilocytes were slight especially since only those cells in the latter stages of crenation bear any similarity to the poikilocytes. The outstanding distinguishing characteristic observed was the regular arrangement of the protrusions from the crenated cell as compared with the haphazard arrangement of those of the poikilocyte.

Most of the studies of poikilocytosis reported in the literature concerned its occurrence in man, and included the condition mainly as a secondary interest in connection with other diseases. Although the deformed red cells have been observed in the blood of several of the lower animals, the reports of the occurrence of these cells in the bovine type of animal are limited to their association with anemia in calves and with anaplasmosis in dairy cattle.

Assuming the normal "in vitro" erythrocyte of dairy cattle to be a circular biconcave disk-shaped cell, many of the bizarre shapes of poikilocytes described in the literature were observed in the blood of 233 of the 423 animals studied during this investigation. Many of the deformed corpuscles were so irregular in shape that no particular name could describe them. The majority of the poikilocytes observed in the blood of the animals severely affected with the disease were generally classifiable in the latter nondescript group of poikilocytes. Deformed cells classed in this category consisted of a

41 Osgood and Ashworth<sup>2b</sup> Da Costa<sup>7a</sup>

body portion from which pseudopodium-like processes protruded which gave the poikilocyte a striking configuration. The degree of cell distortion seemed to be correlated directly with the severity of the condition, the erythrocytes of more severely affected animals manifesting the greater deformation. This observation is in agreement with Da Costa's<sup>7a</sup> finding that the blood of human beings shows poikilocytosis.

In a given vicinity it was found that one entire herd would manifest the condition while a neighboring herd would be completely unaffected. The condition appeared rather widespread, and the incidence of affected animals was high—55.1 per cent of the total number of animals studied.

Although an occasional mature animal manifested poikilocytosis, the young animals and the calves were more frequently affected. Usually health was subnormal in animals whose blood showed 50 per cent or more of the total corpuscles as poikilocytes, and at least 1 animal showed unthriftiness when only 35 per cent of the cells were poikilocytes.

The blood pictures of the affected animals failed to disclose any item that could be associated constantly with poikilocytosis. Anisocytosis appeared frequently in the blood of animals with normal erythrocytes as well as in the blood of those showing poikilocytes and was, therefore, not considered of any consequence. Although the photomicrographs published by Knoop and co-workers<sup>39</sup> indicated the occurrence of poikilocytosis in association with severe anemia in calves, the present investigation revealed that the hemoglobin content, the red blood cell count, the red blood cell volume and the average corpuscular hemoglobin values were independent not only of the incidence of poikilocytosis but also of the degree and severity of cell distortion. The hemoglobin content of the blood of the affected animals ranged from 4.4 to 15.9 Gm per hundred cubic centimeters of blood. The blood of the animal which had the lowest hemoglobin value showed only 25 per cent as many poikilo-

cytes as the animal whose blood had the highest hemoglobin value. It was observed, however, that many of the animals whose blood had a low hemoglobin concentration showed the presence of poikilocytes but that not all of the animals affected with poikilocytosis had a low concentration of hemoglobin in their blood.

In this study observation was made of some dairy herds manifesting poikilocytosis while neighboring herds were unaffected. Since the rations fed to these animals varied greatly in kind and quality, it is assumed that the presence of poikilocytes in the blood was to a large extent attributable to a nutritional deficiency. Some of the dietary factors involved in the control of poikilocytosis in dairy cattle will be presented in a subsequent paper.

#### SUMMARY

Dairy cattle ranging from young calves to mature cows were used in a study of the occurrence and the symptoms of poikilocytosis.

The normal erythrocytes of dairy cattle "in vitro" have a circular biconcave disk shape when observed from a surface view and have the appearance of a dumbbell when observed from an edge view.

The possibility of confusing poikilocytes with the artefacts produced in crenation is negligible.

The distribution of poikilocytes among the 233 affected animals varied up to more than 91 per cent of the red corpuscles (see table 1).

The symptoms manifested by the animals with severe poikilocytosis were anorexia, thinness, unthriftiness, a dry and harsh condition of the hair coat and, in young animals, a retarded rate of growth. Depraved appetite was frequently observed.

The occurrence of poikilocytosis in dairy cattle is independent of the hemoglobin content, the number and the volume of red blood cells and the calcium, inorganic phosphorus and magnesium contents of the blood.



# EXPERIMENTAL REPRODUCTION OF MADUROMYCOTIC LESIONS IN RABBITS

DOUGLAS SYMMERS, M D

General Director of Laboratories, Department of Hospitals, City of New York  
NEW YORK

Up to the present time (1945) twenty-two species of fungi belonging to ten genera and four families have been implicated in the production of the various types of maduromycosis. In spite of this multiplicity of causes the different types clinically may resemble one another closely, and in some instances they may also resemble actinomycosis, especially if the latter disease is confined to a lower extremity as is maduromycosis in many cases. Clinically cases of maduromycosis may be divided into three groups. In all of them the lesions are limited to the part which was originally infected as the foot, the hand, the knee, the neck or the face. Unlike the changes one finds in actinomycosis the deeper viscera seem never to become involved, nor does it appear that maduromycosis has been encountered in cattle or other lower animals. In one group of cases the changes are confined to superficial structures in the form of granulomatous lesions of the skin or of the skin and the subcutaneous tissues. In a second group of cases there are one or more circumscribed fibrous, fatty or, occasionally, cystic nodules usually without ulceration of the overlying skin. In 1 case belonging to this group a solitary tumor-like growth on the dorsum of the foot had been present for eighteen years. The third, and perhaps the commonest variety is characterized by the presence of multiple subcutaneous nodules varying in diameter from one to several centimeters. These nodules are movable, painless and nontender. Spontaneously, or sometimes as the result of injury the superficial nodules rupture and discharge mucopurulent material containing white, yellow or black granules; the color of the granules depending on the type of fungus concerned. Finally the affected part becomes riddled with sinuses which penetrate muscle, fat and even bone.

Symmers and Sporer<sup>1</sup> have shown that there is a series of distinctive histologic changes in a variety of black grain maduromycosis which is

From the Laboratories of Pathology, Goldwater Memorial Hospital

<sup>1</sup> Symmers, D, and Sporer, A. *Arch Path* **37** 309, 1944

caused by the presence in the tissues of a fungus composed of septate and branching mycelial filaments and chlamydospores, the latter predominating. They described three types of granuloma of different degrees of maturity occurring in the midst of cellular and well vascularized granulation tissue which is richly infiltrated by foam cells. The youngest granulomas consist almost exclusively of clumps of brownish black chlamydospores embedded in accumulations of polymorphonuclear neutrophilic leukocytes. The older granulomas are more or less sharply circumscribed by connective tissue and most of them are arranged around collections of multinuclear giant cells containing phagocytosed necrotic chlamydospores. In the case recorded by Symmers and Sporer efforts to cultivate the fungus on various artificial mediums were continued over a period of several months but were unsuccessful. The object of the present paper is to note the results of subsequent successful efforts to cultivate the causative fungus from the tissues of the same patient and to describe the changes produced by inoculating the subcutaneous tissues of rabbits with black grains freshly removed from the patient's hand and with the fungus that was grown on artificial culture medium. The patient from whom the fungus was isolated was a white man aged 67, a clerk, who stated that in 1935, two years before admission to the hospital, he fell and scraped his right hand on an old and partially decayed wooden floor, "picking up many splinters." Two or three weeks later the right hand became swollen and multiple pustules appeared with black dots in them. At the time of the patient's admission the hand was greatly swollen and showed many nodular formations on both the palmar and the dorsal aspect. Some of these nodules contained sinuses which discharged yellowish pus containing black granules. The causative fungus was recently identified by Dr. C. W. Emmons<sup>2</sup> as an organism which was first described by Langeron<sup>3</sup> in 1928 under the name of

<sup>2</sup> Emmons, C. W. Personal communication to the author

<sup>3</sup> Langeron, M. *Ann de parasitol* **6** 385, 1928

*Torula jeanselmei* and was isolated from maduromycosis of the foot. However, Dr. Emmons regards the fungus as a species of *Phialophora* and transfers it to that genus under the designation of *Phialophora jeanselmei*.

#### EXPERIMENTAL INOCULATION OF RABBITS

Granules of fungus that had been freshly removed from the hand of a patient with maduromycosis or that had grown after inoculation on artificial culture medium (Sabouraud's agar with 6 per cent dextrose and liver extract) were crushed in sterile salt solution and injected into the subcutaneous tissues of rabbits just below the level of the interscapular fat pads. This method of introduction was selected in order to duplicate as nearly as possible the route followed by the fungus when man is infected through the skin by pricks of contaminated thorns or as the result of other superficial and apparently trivial injuries. Each rabbit was inoculated on only one occasion and at one point only, although liberal quantities of fungus were distributed through the point of injection into different areas covering a radius of 4 or 5 cm. It was thought that local reactions would occur resulting in the formation of multiple nodules corresponding to the places where the fungus had been deposited. In this regard the results were disappointing. The injected granules seemed invariably to gather at a common point so that in each rabbit only a single nodule occurred. The nodules were excised, with the animal under local anesthesia, at intervals varying from three to one hundred and twenty-six days. The tissues were fixed in 4 per cent formaldehyde solution and embedded in paraffin. Microscopic examination showed essentially the same changes as those described by Symmers and Sporer in a variety of human maduromycosis. As in man, the lesions in the rabbit consisted of localized granulomas of different degrees of maturity displayed against a background of moderately well vascularized and cellular or irregularly sclerotic granulation tissue. In those nodules which followed injections of fungus directly removed from the patient the granulation tissue contained great numbers of foam cells, and the granulomas were formed around necrotic and fused chlamydo-spores together with occasional necrotic mycelial filaments thus duplicating the histologic changes described by Symmers and Sporer in the tissues of a human patient with maduromycosis. On the other hand, in those nodules which were produced experimentally in rabbits by the artificially grown fungus, the granulomas were formed around apparently well preserved mycelial filaments and chlamydo-spores.

Reifenstein, Ferguson and Weiskotten<sup>4</sup> have shown that in physiologic circumstances the lymphocyte is the prevailing cell in the rabbit's blood, polymorphonuclear neutrophils occurring in relatively small numbers. Eosinophils are present in practically the same numbers as in the blood of man. From this it follows as a matter of no surprise that the earliest inflammatory changes in experimental maduromycotic lesions in rabbits are attended by preponderant exudation of lymphocytes, although in both the acute exudative and the chronic productive inflammatory lesions it is astonishing to note how often there are great numbers of eosinophils.

For purposes of comparison, rabbits were inoculated with artificially grown fungus that had been suspended

in salt solution and autoclaved for fifteen minutes on three successive days at 260 C under a pressure of 20 pounds (9 Kg). The structure of the autoclaved fungus appeared to be intact, and cultures were negative. During that period of experimentation on rabbits when it was desired to inject granules that had been removed directly from the patient and killed by autoclaving, no granules were available because all the sinuses in the patient's hand were closed and had apparently healed completely.

Granules that had been freshly removed from the sinuses in the patient's hand were injected into rabbit 1 April 26. On June 23, fifty-eight days after this injection, a solitary subcutaneous nodule had attained the size of 0.7 by 0.3 by 0.3 cm. The nodule was elevated above the surface of a fat lobule to which it was attached and was well circumscribed. It was faintly yellowish and speckled with black. Occasional black specks lay free in the surrounding fat tissues without visible evidence of reaction around them. Microscopically, the nodule was surrounded by moderately well vascularized and cellular connective tissue, which in one or two places enclosed small lymph follicles. The body of the nodule was composed of irregularly sclerotic granulation tissue in which there were numbers of eosinophils, many fibroblasts, a sprinkling of lymphocytes, innumerable foam cells and circumscribed granulomas in different stages of maturity. The youngest granuloma was made up of collections of dark brown, degenerate and clumped chlamydo-spores, along the edges of which were pinkish-staining short projections representing necrotic mycelial remnants, the whole embedded in collections of lymphocytes, polymorphonuclear neutrophilic leukocytes and eosinophils, many of them necrotic (fig 1). In other parts of the nodule there were numbers of rounded or oval mature granulomas which were encapsulated by fibroblastic connective tissue, enclosing variously sized clumps of similarly pigmented and necrotic chlamydo-spores and numbers of eosinophils. In some of the encapsulated, mature granulomas there were multinuclear giant cells, which showed phagocytosed chlamydo-spores in their cytoplasm (fig 2). In other words, histologically, both the non-encapsulated, immature and the encapsulated, mature granulomas were practically identical with the same sort of granulomas which occur in the tissues of human patients with maduromycosis.

Two cubic centimeters of a suspension of living, artificially grown fungus was injected into rabbit 2 June 7. The animal died three days later. At necropsy there were two small clumps of blackish material near the point of injection. The tissues in the immediate vicinity appeared to be edematous. Otherwise necropsy revealed nothing of note in the present connection. Microscopic examination showed several large and small, rounded, oval or irregularly outlined clumps of brownish, apparently well preserved fungus surrounded by mantles of lymphocytes. Beginning encapsulation was indicated by the presence of a delicate but incomplete layer of fibroblastic connective tissue (fig 3). The granulation tissue in the immediate proximity of most of the granulomas was edematous and contained scattered collections of fungus with no indication of reaction around them.

Two cubic centimeters of a suspension of living, artificially cultivated fungus was injected into rabbit 3. June 6. June 21, fifteen days after the injection, a nodule measuring 0.5 by 0.5 by 0.5 cm was removed from the subcutaneous tissues. It was freely movable and sharply circumscribed. On section the center of the nodule was composed of whitish, glistening firm

<sup>4</sup> Reifenstein, G. H., Ferguson, J. H., and Weiskotten, H. G. *Am J Path* 17:219, 1941.

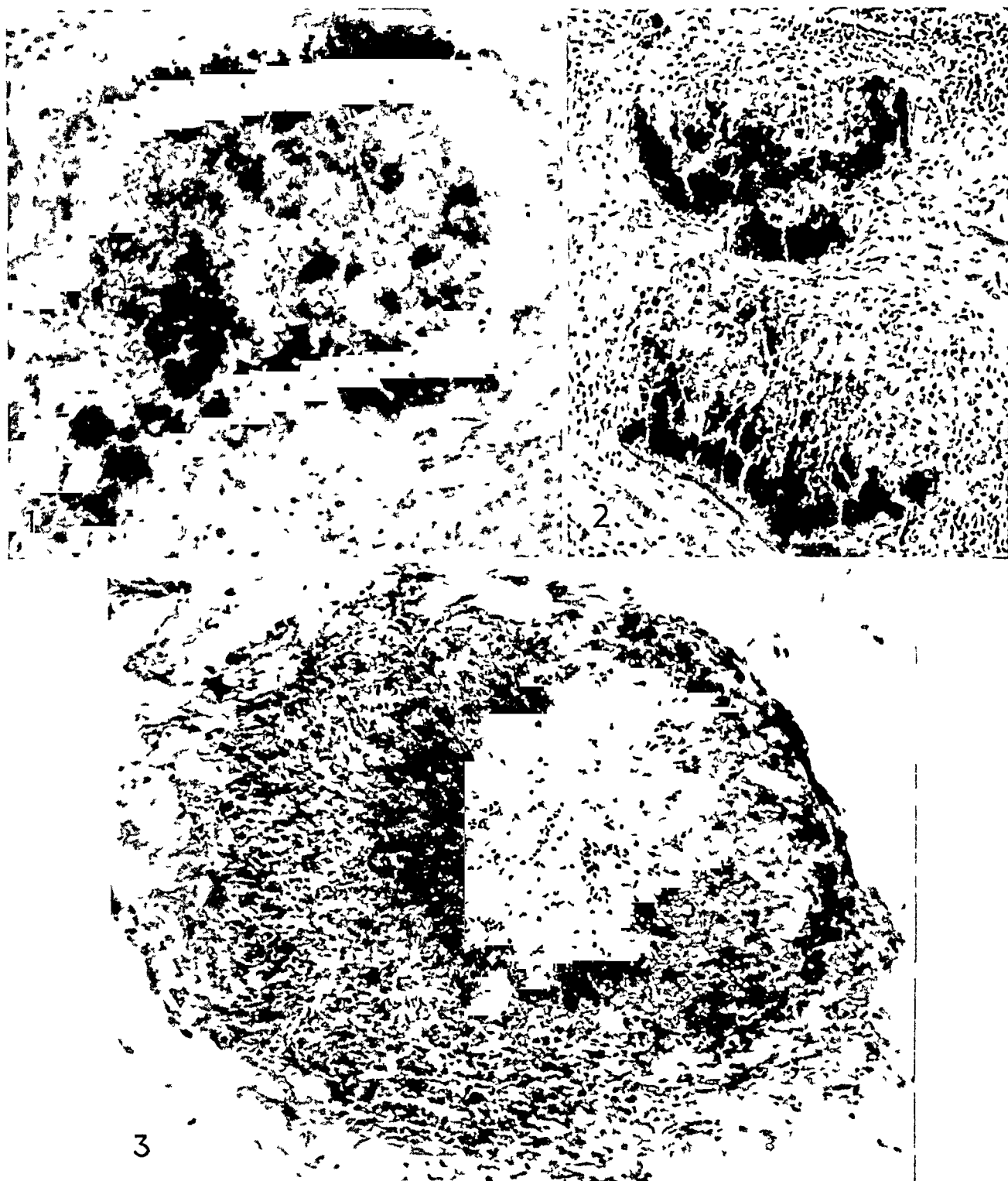


Fig 1—Photomicrograph of an immature granuloma showing an irregularly outlined clump of pigmented chlamydospores and, at the periphery, numerous lightly staining short projections representing necrotic mycelia, the whole embedded in a matrix of leukocytes. From rabbit 1, inoculated with black granules freshly removed from a patient with maduromycosis of the hand, hematoxylin and eosin, paraffin section,  $\times 396$ .

Fig 2—Photomicrograph showing two mature granulomas with clumped and pigmented chlamydospores. The lower granuloma is almost completely encapsulated and shows multinuclear giant cells with phagocytosed chlamydospores. From rabbit 1 inoculated with black granules freshly removed from a patient with maduromycosis of the hand, hematoxylin and eosin, paraffin section,  $\times 200$ .

Fig 3—Photomicrograph of an immature granuloma showing a central collection of chlamydospores, short mycelia at the periphery and an admixture of lymphocytes. From rabbit 2 inoculated with living, artificially grown fungus, hematoxylin and eosin, paraffin section,  $\times 316$ .

tissue while the periphery was light brown and numerous minute black specks were visible in it. Microscopic examination showed an edematous and delicate fibrillar connective tissue capsule. At one side of the nodule, immediately under the capsule, were large collections of lymphocytes scattered through which were clumps of brownish, apparently well preserved fungous cells. To the inner side there was a large field made up of granulation tissue which in places was richly vascularized by thin-walled capillary blood vessels lying in collections of eosinophils and moderate numbers of lymphocytes. In other places the granulation tissue was traversed by intercommunicating bands of poorly cellular, occasionally hyalinized connective tissue surrounding islands made up of capillary blood vessels, numerous eosinophils and a few lymphocytes. In one part there was a large granuloma which was circumscribed by fibrillar connective tissue. The body of the granuloma was made up of a central collection of eosinophils, while in the immediate vicinity was a solitary multinuclear giant cell rimmed at one edge by a layer of brownish phagocytosed fungous cells (fig 4). Lying free in the tissues of the granuloma was a small mass of apparently well preserved fungus. In still other places the granuloma contained several non-nucleated large protoplasmic bodies representing, probably, immature giant cells.

A suspension of living, artificially cultivated fungus was injected into rabbit 8 June 6. July 10 it was noted that there was a freely movable nodule immediately beneath the skin near the point of injection. The nodule measured approximately 0.5 cm in length and was blackish. The mass was removed August 2, fifty-seven days after the injection. On removal the nodule was oval, measured 1 by 0.4 by 0.4 cm and was speckled with black. It was surrounded by a smooth, glistening capsule. In the immediate vicinity were several minute black granules which lay free in the tissues without any obvious reaction around them. Microscopically, the nodule was surrounded by a thin capsule of mature connective tissue. The body of the nodule was made up of numerous large and small clumps of fungus consisting of apparently well preserved brownish chlamydo-spores and short segmented and occasionally branching mycelial filaments, likewise brownish, lying among masses of necrotic lymphocytes.

Large quantities of a suspension of living, artificially grown fungus were injected September 26 into rabbit 12. January 30, one hundred and twenty-six days later, a subcutaneous nodule was removed. It measured 0.8 by 0.8 by 0.4 cm, and, on section, was composed of an outer smooth whitish hyaline layer and a core of yellowish granular material, each measuring about 0.2 cm in thickness. No black granules were visible to the unaided eye. Microscopically, the outer part of the nodule was composed of thick hyalinized connective tissue, beneath which there were large numbers of fibroblasts and foam cells. Frozen sections stained with sudan IV showed numerous fat globules in the cytoplasm of many of the fibroblasts and in all of the foam cells, the latter cells were obviously derived from the former. The center of the nodule was composed of necrotic lymphocytes, among which were variously sized clumps of apparently well preserved fungous cells lying free or enclosed in the cytoplasm of multinuclear giant cells.

Large quantities of artificially grown fungus which had been suspended in salt solution and killed by autoclaving were injected into rabbit 6 June 28. July 1 a quantity of blackish tissue measuring 0.6 by 0.4 by 0.3 cm was removed. Microscopically it was com-

posed of variously sized collections of well preserved brownish fungous cells embedded in large accumulations of lymphocytes. In some of the serial sections there was a solitary immature small granuloma which contained a central collection of fungous cells surrounded by a mantle of lymphocytes and, at the periphery, by a delicate layer of fibrillar connective tissue, the whole corresponding histologically to the youngest form of granuloma encountered in human maduromycosis and to the experimentally produced granuloma in rabbit 2.

A suspension of killed fungus was injected into rabbit 11 July 28. September 20, fifty-four days later, a nodule was removed which measured 1 by 1 by 0.3 cm. The nodule was covered by a filmy connective tissue capsule and was freely movable, it was mottled in pink, black and yellow, lobulated, tense in consistency and flattened. Microscopically, it was surrounded by an irregularly vascularized fibrillar connective tissue capsule. The body of the nodule was composed almost exclusively of rather large rounded cells with poorly chromatic, vesicular nuclei and pinkish-staining granular or finely reticulated cytoplasm, morphologically suggesting foam cells in miniature. They were divided into islands of irregular shapes and sizes by intercommunicating bands of moderately well vascularized connective tissue. In many of the islands, groups of eosinophils were to be seen with or without polymorphonuclear neutrophilic leukocytes. Others enclosed collections of apparently well preserved brownish fungous cells (fig 5). In still others, groups of calcified chlamydo-spores were present.

#### COMMENT

One of the advantages of reproducing human disease in lower animals is to provide a means for experimental methods of treatment. It is believed that the histologic changes in that form of maduromycosis which is due to *Phialophora jeanselmei* have been sufficiently faithfully reproduced in rabbits to furnish an acceptable approach for the use of experimental methods of therapy. In this form of maduromycosis the granules that are discharged from the sinuses in diseased tissues as well as the colonies which grow on artificial culture mediums are composed of apparently well preserved mycelial filaments and chlamydo-spores. In microscopic preparations of diseased tissues the morphologic aspect of the fungus is noticeably changed, the organism occurring almost exclusively in the form of disintegrated chlamydo-spores. Mycelia, if present at all, are practically always necrotic. When granules are removed directly from the discharging sinuses of the patient and are injected subcutaneously into rabbits, the fungus appears in the rabbits' tissues in the form of similarly disintegrated chlamydo-spores with or without necrotic mycelia. On the other hand, when the artificially grown fungus is injected into rabbits, the structure of the fungus remains apparently unchanged. In those instances in which the organism is destroyed in the tissues it is not evident whether destruction follows devitalization or whether the reverse is true. However this may be it is ob-

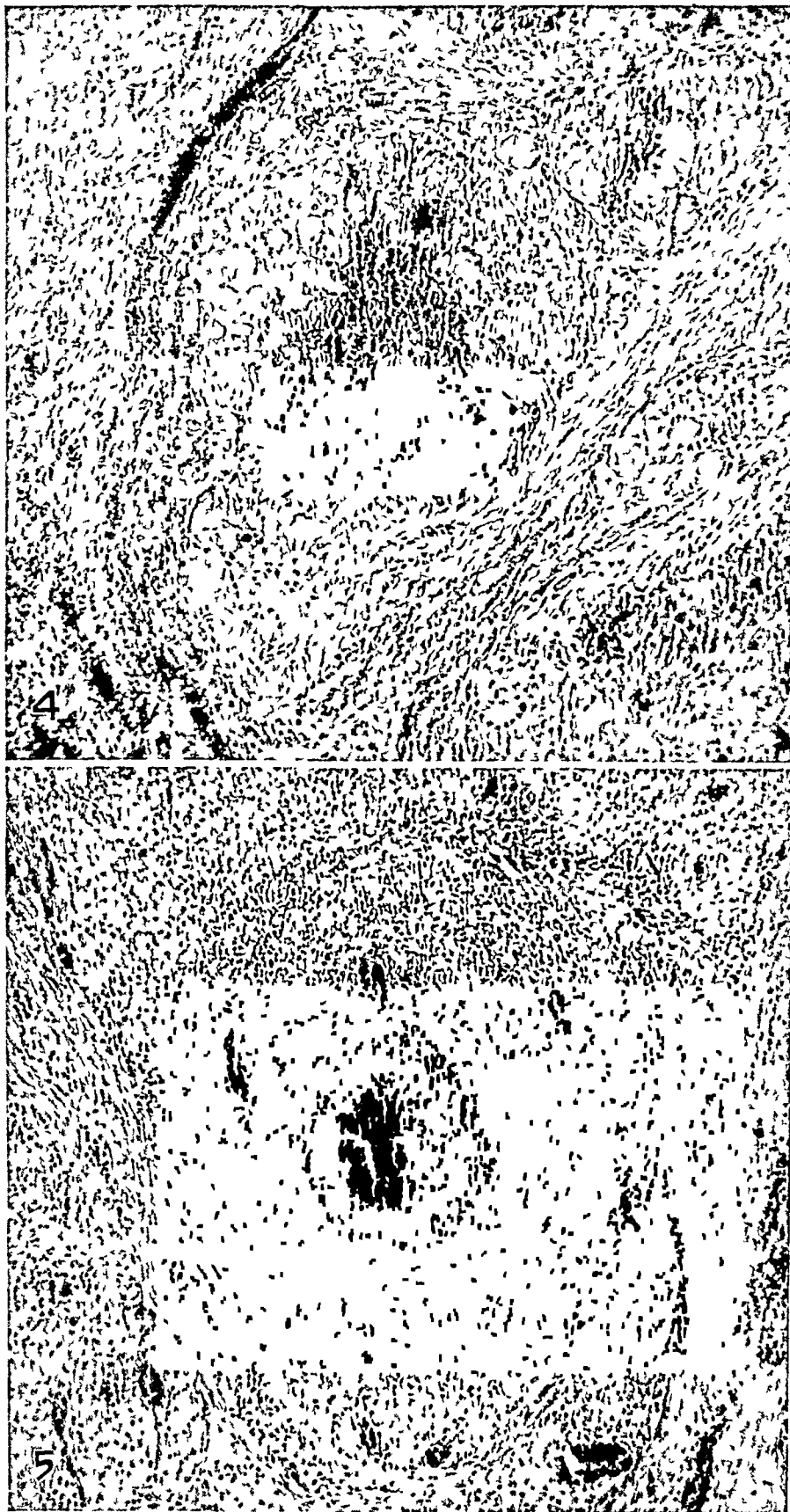


Fig 4—Photomicrograph showing mature, encapsulated granulomas. The partially circumscribed granuloma at the left contains a large central collection of eosinophils and at the lower right a multinuclear giant cell rimmed below by phagocytosed fungous cells. From rabbit 3, inoculated with living, artificially grown fungus, hematoxylin and eosin, paraffin section,  $\times 135$ .

Fig 5—Photomicrograph showing a solitary, partially encapsulated, mature granuloma. At the center is a clump of fused fungous cells lying in a collection of eosinophils. The greater part of the body of the granuloma is composed of lightly staining cells suggesting miniature foam cells. From rabbit 11, inoculated with killed, artificially grown fungus, hematoxylin and eosin, paraffin section,  $\times 90$ .

vious that in order to determine experimentally the value of any therapeutic agent for use in this form of maduromycosis the source of the fungus must be known, that is to say, whether it is derived from diseased tissues or from artificial culture mediums

In approaching the treatment of maduromycotic lesions in experimentally infected rabbits it seems logical to attempt first to learn the effect of various supposedly injurious agents on the fungus *in vitro*. It has already happened several times in my experience that the fungus remained alive and its structure apparently intact after prolonged exposure to substances of this description. In another instance the fungus remained morphologically intact but had apparently been de-

vitalized, since it could not be grown subsequently on artificial culture mediums. At all events, these are the general lines of procedure that are now being followed in efforts to determine the value, if any, of various substances in the treatment of experimentally produced maduromycotic lesions in rabbits.

#### CONCLUSION

*Phialophora jeanselmei* when injected subcutaneously into rabbits produces solitary non-ulcerative nodules which histologically are specific and are closely comparable to the nodules in that form of maduromycosis in man which is caused by the same fungus.

# PHIALOPHORA JEANSELMER COMB N FROM MYCETOMA OF THE HAND

C W EMMONS, PH D

Principal Mycologist, United States Public Health Service

BETHESDA, MD

The term "mycetoma" was proposed by Carter<sup>1</sup> to designate fungous infections of the subcutaneous tissues characterized by swelling, formation of sinuses and production of pus in which there are well organized mycotic granules. The granules differ in size, shape, hardness and color and in the morphology of the constituent hyphae, as Carter<sup>2</sup> pointed out. As additional cases were investigated it became apparent that although the cases had certain clinical characteristics in common actually there are several types of mycetoma, depending on the identity of the etiologic agents. The relationships of several species of fungi to cases of mycetoma were elucidated by Brumpt,<sup>3</sup> and Pinoy<sup>4</sup> proposed a division into the actinomycoses caused by species of *Actinomyces* and *Nocardia*, and the "true mycetomas" caused by fungi of several unrelated genera having in common only the characteristic of larger hyphae than are found in *Actinomyces*. Chalmers and Archibald<sup>5</sup> substituted the name "maduromycosis" for "true mycetoma," and this name has been used widely. Some writers, however, have used it as a synonym of "mycetoma" without recognizing the distinction made by Chalmers and Archibald.

The choice of the name "maduromycosis" to designate the latter group of "true mycetomas" is perhaps unfortunate because of the derivation of the word and the heterogeneity of the tumors in the group. According to Carter,<sup>2</sup> Colebrook introduced into scientific literature the name "Madura foot," by which mycetoma of the foot was locally known near Madura, India and Brumpt<sup>3</sup> created the name *Madurella* for a fungus causing it. It is apparent, however, from Carter's reports

that from the earliest studies a multiple causation was recognized and that species of *Nocardia* were among the fungi causing Madura foot. Subsequently the term "Madura foot" has been used rather loosely to designate mycetoma of the foot in cases of diverse geographic origin and cause. Nine species of *Madurella* have been described, and besides these there are species in several other genera which cause similar tumors of the foot. The latter fungi appear to be mycologically unrelated to *Madurella* (which may be itself a heterogeneous aggregation), and the lesions they cause are not all like those of the originally described Madura foot. It may be questioned whether the term "maduromycosis" designates this heterogeneous group any more conveniently than the original name, "mycetoma." Neither term is precise and both require supplementary labeling as to etiology in order to convey information about the true nature of a given case. If "maduromycosis" is used it should be used in the restricted sense and not as a synonym of "mycetoma."

Gammel<sup>6</sup> summarized more recent knowledge about the fungi causing the tumors grouped as mycetoma and described as new two species which he had studied. He remarked that mycologic knowledge about the fungi causing mycetoma was meager. Unfortunately, this is still true, owing in part to the fact that adequate mycologic studies have not been made in many cases, and in part to the fact that these fungi sometimes grow abnormally on agar culture mediums not revealing their actual mycologic relationships. These fungi need to be restudied with special attention to the variability of strains and the correct interpretation of abnormal or equivocal structures. This may make it possible, on the one hand, to reduce to synonymy some of the many species names now associated with these infections and, on the other, to determine the relationships between genera in which these species

From the Division of Infectious Diseases, National Institute of Health.

1 Carter, H. V. Tr. Med-Phys. Soc. Bombay (1861) 7: 206, 1862.

2 Carter, H. V. Tr. Med-Phys. Soc. Bombay (1860) 6: 104, 1861.

3 Brumpt, E. Arch. de parasitol. 10: 489, 1906.

4 Pinoy, E. Bull. Inst. Pasteur 11: 929 and 977, 1913.

5 Chalmers, A. J. and Archibald, R. G. Ann. Trop. Med. 10: 169, 1916.

6 Gammel, J. A. Arch. Derm. & Syph. 15: 241, 1927.



are now placed. Recent observations<sup>7</sup> have shown, for example, that *Allescheria boydii* is the ascocarpic form of *Monosporium apiospermum* (one of the most frequent causes of mycetoma in the United States). The relationship between these two forms had been suspected, but direct proof of it had not been presented previously.

Symmers and Sporer<sup>8</sup> recently reported a case of mycetoma of the hand. The fungus was isolated by Symmers and used by him in experimental inoculation of rabbits, as reported in a paper<sup>9</sup> accompanying this one. The fungus is unlike those isolated in most cases of mycetoma. However, it resembles closely a fungus which was

small many being hollow with irregular interruptions of the wall, as shown in sections (figs 3 and 4 of Symmers and Sporer, and figs 2 and 6 of Langeron). Chlamydospores were conspicuous features of the granules in both cases. In most cases, on the contrary, the granule is firm, with a relatively dense structure composed of radiating hyphae. Exceptions have been listed by Langeron. Clinical and histologic similarities in themselves are not conclusive evidence that the 2 cases had a common etiologic factor. It is generally conceded that the fungus causing mycetoma in any case can be identified only after its isolation in pure culture. The characteristics of

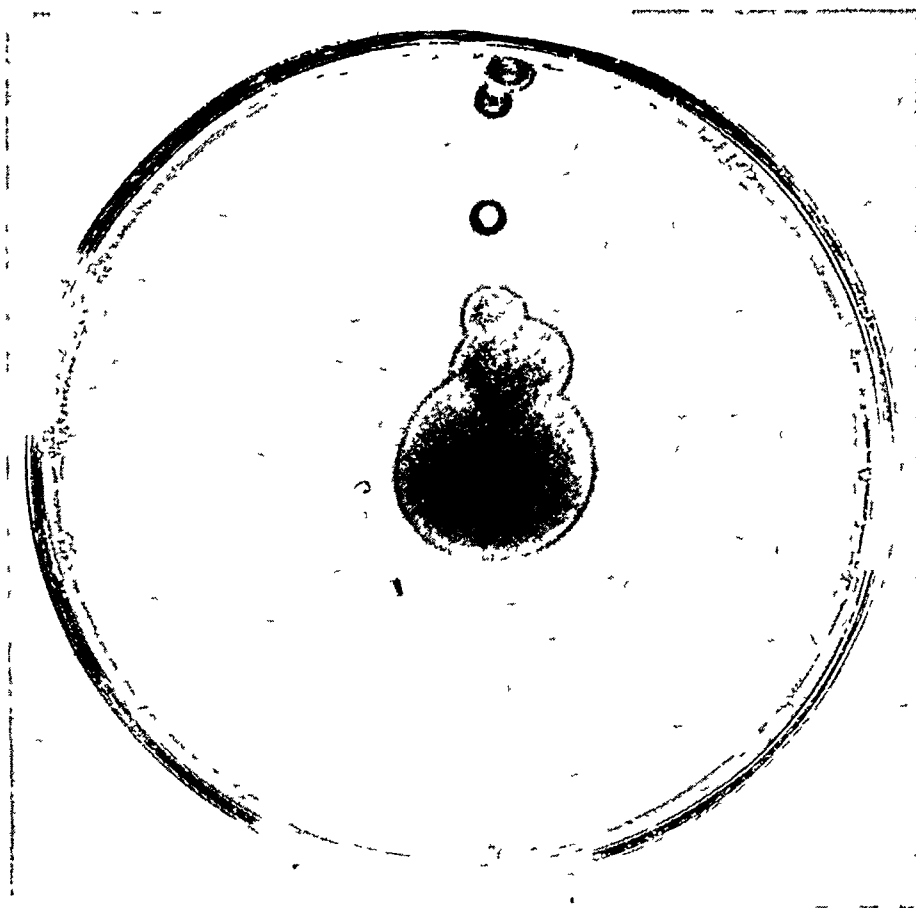


Fig 1—A colony of *Phialophora jeanselmei* comb n grown on modified Sabouraud's agar

described as *Torula jeanselmei* by Langeron<sup>10</sup> after its isolation in a case of mycetoma reported by Jeanselme, Huet and Lotte<sup>11</sup>. The clinical changes in the latter case closely resembled those reported by Symmers and Sporer except that the foot rather than the hand was involved. In both cases the granules were unlike those seen in most instances of mycetoma. They were relatively

the fungus isolated from the patient observed by Symmers and Sporer correspond closely to those of the fungus which Langeron described. This fact together with the clinical and pathologic similarities, has led to the conclusion that the two strains are identical.

The fungus described in this paper when grown on modified Sabouraud's agar (Difco neopeptone 1 per cent, chemically pure dextrose 2 per cent) produces a colony which is dome shaped, with an entire margin reaching a diameter of 22 mm and a height of 5 mm after two weeks' growth at 30 C (fig 1). The color is

<sup>7</sup> Emmons C W. *Mycologia* **36** 188, 1944.

<sup>8</sup> Symmers D, and Sporer, A. *Arch Path* **37** 309, 1944.

<sup>9</sup> Symmers, D. *Arch Path* this issue, p 358.

<sup>10</sup> Langeron, M. *Ann de parasitol* **6** 385 1928.

<sup>11</sup> Jeanselme Huet and Lotte. *Bull Soc franç. de dermat et syph* **35** 369, 1928.

"deep grayish olive" <sup>12</sup> The surface is covered with delicate short aerial hyphae, giving the colony a fine plushlike appearance

On glycerin agar slants after two weeks at 30 C a colony 1 to 1.5 mm wide appears along the line where spores were deposited at the time of inoculation, and colonies reaching a diameter of 5 mm develop where larger pieces of inoculum lodged. These colonies are nearly black and appear at first glance to be glabrous, but careful examination with a lens shows numerous coremia or tapering hyphal aggregations rising from the surface. The growth on horse meat infusion agar is similar. In gelatin after two weeks' growth at 30 C minute colonies develop at the bottom of the tube, but no liquefaction has been observed.

The fungus grows poorly on corn meal agar. Some transfers failed to make any visible growth, and others developed into very small, black, nearly glabrous colonies, almost microscopic in size. No growth was apparent after two weeks at 30 C on glycerinated potato plugs and litmus milk. Indole was not formed.

Growth was better at 30 C than at either room temperature or 37 C.

Microscopic examination reveals dark-colored septate hyphae varying in diameter from 1 to 3 microns (fig 2 A to F). Many hyphae, especially those which first develop are toruloid (fig 2 G to I). The conidiophores are tubular (fig 2 A, B, E and F) or flask shaped (fig 2 C and D) and stand at an angle of 90 degrees or less from the vegetative hyphae. Many are single and unbranched, but others are branched and may occur in groups. Some are tubular and of nearly uniform diameter to a point near the distal end, where they taper to a narrow tip (fig 2 A, B, E and F). Others are urn shaped, tapering toward both the base and the tip (fig 2 C and D). In some cases the tip extends into a flaring cup (fig 2 C). The dimensions of the conidiophores are too variable to be of much value in identifying the fungus, but the tubular conidiophores are usually 1 to 3 microns in diameter and 5 to 15 microns long. In addition to the morphologically well differentiated conidiophores, many hyphae bear conidia laterally on very short rudimentary conidiophores (fig 2 E), which in many cases are little more than pegs or apiculate knobs on the hyphal walls. In some cases this method of sporulation recalls that found in *Pullularia pullulans*.

On all of the types of conidiophores described here the conidia are borne at the tip. They are pushed out serially through an opening at the end of the conidiophore, where they collect in a group held together by some adhesive substance. The conidia are hyaline or subhyaline, elliptic, and about 0.75 to 1 by 1 to 2 microns in size. Conidia which are borne at the agar surface or which come into contact with it continue to grow, reaching a size of 2 to 3 by 4 to 5 microns or larger. The walls of these large conidia contain the same brown pigment which colors the hyphae and the conidiophores. When conidia at the agar surface increase in size, they usually bud in a yeastlike manner to form secondary conidia.

Despite the tendency of conidia to cluster about the tips of conidiophores it is apparent that when the mycelium is disturbed some conidia are readily discharged from it. If, in transferring a culture one picks up a portion of dry sporulating mycelium and attempts to place it at the center of an agar plate, spores almost invariably are dropped along the way from the edge to the center of the Petri dish (fig 1). The actual mechanism of this spore discharge has not been determined.

In attempting to identify this fungus one is struck at once by the resemblance of a colony to that of *Phialophora verrucosa*, an etiologic agent of chromoblastomycosis. The color of a colony grown on modified Sabouraud's agar is practically identical with that of a colony of the latter fungus but the rate of growth is slower, and this slow rate of growth is even more apparent on corn meal, Czapek's and glycerin agars. There is also a microscopic resemblance between the two species with respect to the brown vegetative hyphae and the urn-shaped conidiophore with flaring mouth. However, most of the conidiophores lack this phialoform structure for which the genus was named. It may be mentioned incidentally that this structure is also poorly developed or sometimes lacking in some other species of the genus isolated from wood and originally described as species of *Cadophora*. Despite a superficial resemblance to *P. verrucosa* the fungus can be differentiated easily from that species by the greater variability of its conidiophores, the somewhat smaller size of the young conidia, a greater tendency of the conidia to produce secondary conidia and its poor growth on certain mediums already mentioned, on which *P. verrucosa* grows readily. Furthermore, *P. verrucosa* grows in human tissue in the form of chlamydospores aggregated at most into small clusters, whereas this fungus forms granules a millimeter

<sup>12</sup> Ridgway, R. Color Standards and Color Nomenclature, Washington, D. C., The Author, 1912

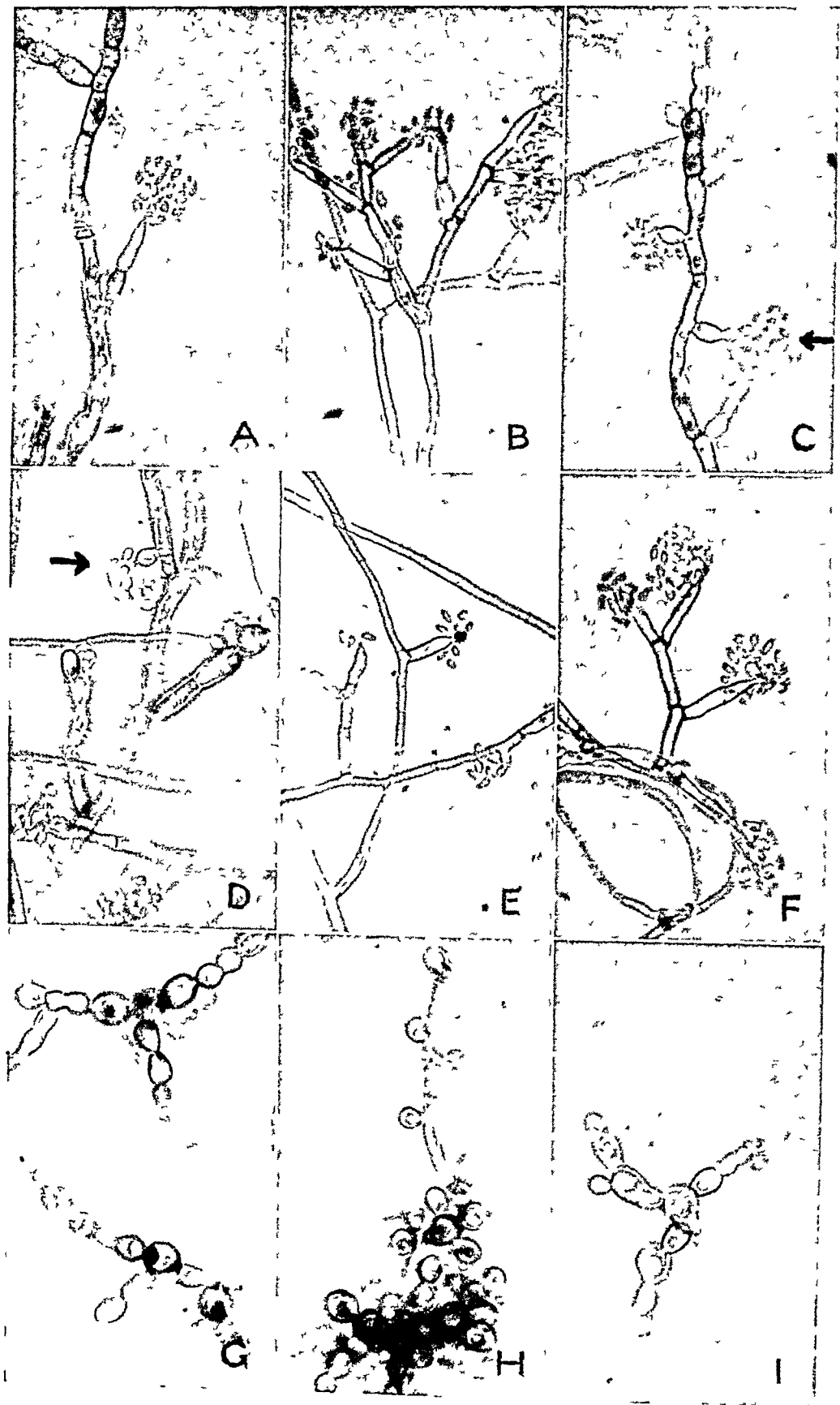


Fig 2—Various appearances of *Phialophora jeanselmei*. In C and D arrows indicate the two types of conidiophores observed—that discharging conidia through a flaring cuplike structure and that discharging them through a short tubelike extension

or two in diameter. It can be pointed out, however, that in these granules in both species chlamydospores are conspicuous features and that the transition from a cluster of several chlamydospores (as in chromoblastomycosis) to a macroscopic granule (as in this type of mycetoma) would result if there were more rapid growth and cell division and a greater degree of adhesion between cells in the case of the larger granule. It therefore appears proper to place this fungus in the genus *Phialophora*, but it does not correspond precisely to any species which have been found described within that genus.

In all respects the fungus appears to be like the one Langeron described as *Torula jeanselmei* and this resemblance clearly appears in a comparison of the illustrations in figure 2 with the illustrations Langeron published as figures 8 to 12. In both fungi the growth begins as a cluster of toruloid hyphae, many of which, as they develop, bear lateral buds and spores which are not associated with morphologically differentiated conidiophores (fig 2 I). In older cultures tubular conidiophores are more clearly differentiated from the vegetative hyphae, and they bear terminal clusters of spores. Langeron did not illustrate the cluster of conidia at the tip of a conidiophore, but the probable reason for this appears from a study of our fungus. There is little adhesion between these conidia, and if mycelium containing these clusters of conidia is teased out in the usual manner for microscopic examination the conidia almost invariably float away. The relationship illustrated in figure 2 A to F can be demonstrated only by a careful examination of slide cultures.

The conidiophores illustrated in Langeron's figures 11 and 12 are apparently of the type illustrated here in figure 2 C and D. Langeron's figure 12a illustrated the budding of conidia to

form secondary conidia, which is commonly seen in our strain. He did not illustrate conidiophores with flaring mouths. Although a rare conidiophore of this type, bearing an expanded apical structure as illustrated in figure 2 C, was found in our strain many have only a very delicate tubular extension through which the spores are pushed out (fig 2 D), and there is little apparent morphologic differentiation of this type at the fertile tip of most conidiophores. The resemblances between Langeron's fungus and the one described in this paper are so close that they are considered identical.

Langeron described his fungus as a new species of *Torula* placing it in that genus because of the budding nature of the conidia and, presumably, because of the toruloid character of many hyphae. These are rather indefinite characteristics for the correct allocation of a species if other more definitive characteristics can be found. The types of conidiophores illustrated by Langeron (figs 11 and 12) suggest the genus *Phialophora* rather than *Torula*. The species is therefore transferred to that genus under the name *Phialophora jeanselmei* (Langeron) comb n.

The fungus has been entered in the American Type Culture Collection where it is listed as no. 9541.

#### SUMMARY

The mycologic characteristics of a fungus isolated from mycetoma of the hand are given. The condition developed after an injury in which splinters from a wooden floor pierced the skin. The fungus resembles *Phialophora* which is known to occur on wood. It is specifically identified with *Torula jeanselmei* Langeron which was isolated from mycetoma of the foot. This species is transferred to *Phialophora*, the name becoming *Phialophora jeanselmei* comb n.

# ACUTE AGRANULOCYTOSIS

A MICROSCOPIC STUDY OF ULCERONECROTIC GINGIVA AND OF BONE MARROW IN A CASE

CAPTAIN GEORGE J ANDAY  
MEDICAL CORPS, ARMY OF THE UNITED STATES  
AND  
BALINT ORBAN, M D, D D S  
CHICAGO

Gingivitis and stomatitis are among the earliest and most common symptoms of agranulocytosis. Necrotic ulcers occurring at the margins of the gingiva are frequently encountered. Jackson, Parker, Rhinehart and Taylor<sup>1</sup> observed oral lesions in each of 13 patients whom they studied. Lichtenstein<sup>2</sup> reported 27 cases of agranulocytosis, in 17 of which there were oral lesions. Reviewing 43 cases from the literature Kastlin<sup>3</sup> noticed an inflammatory process of the oral lining in all cases and necrotic ulcers of the gingiva in 9. The early appearance of gingivitis is remarkably well demonstrated in a case of recurring agranulocytosis observed by Cook<sup>4</sup>, each of his patient's four subsequent attacks started with gingivitis. For compiled data on oral lesions in agranulocytosis the reader is referred to the comprehensive reviews by Appleton,<sup>5</sup> Cahn,<sup>6</sup> Epstein,<sup>7</sup> Hanzlik<sup>8</sup> and Watkins,<sup>9</sup> as well as to the standard textbooks on dentistry.

Despite their frequency, histologic examination of gingival lesions is almost entirely lacking. Most reports mention only the gross lesions. Those reports containing histologic data usually give a general description of the necrotic lesions of the entire oral or oropharyngeal lining without specific reference to the gingiva. One of the

most lucid histologic descriptions of a necrotic ulcer on the posterior pharyngeal wall is that of Ophuls, of forty-three years ago, reported in the frequently quoted paper of Brown.<sup>10</sup> Several similar descriptions have been recorded since the clinical picture of agranulocytosis was described by Schultz in 1922. Apart from the characteristic necrosis, lack of polymorphonuclear leukocytes and edema little attention was paid to the differences in the cellular reaction of the tissues around the lesion. In Brown's<sup>10</sup> report the zone of infiltration around the necrosis was said to consist mostly of "epitheloid" cells, some lymphocytes and few polymorphonuclear leukocytes. A few similar cells but no particular zone of reaction was observed around the oropharyngeal lesions by Lovett.<sup>11</sup> Kastlin<sup>3</sup> and Rotter<sup>12</sup>. Marked lymphocytic and plasma cell infiltration was present around the necrosis in a case reported by Rotter<sup>12</sup> (tonsils) and in Plum's<sup>13</sup> cases ("neck organs"). Petri<sup>14</sup> observed in extensive ulcerations of the gastrointestinal tract lymphocytes, some histiocytes, plasma cells and large "lymphocyte-like" cells.

The present histologic study was made on a biopsy specimen of the gingiva in a case of acute agranulocytosis. The advantages of a fresh biopsy specimen for finer histologic study are evident. Nevertheless, only one reference to a biopsy of the gingiva in the literature appears to be available, that of Bernier,<sup>15</sup> although a detailed analysis of his case is lacking. As far

From the Department of Medicine, Loyola University School of Medicine (Captain Anday) and the Research Department of the School of Dentistry of Loyola University, Chicago College of Dental Surgery (Dr Orban).

1 Jackson, H, Jr, Parker, F, Rhinehart, J, and Taylor, F H. *J A M A* **97** 1436, 1931.

2 Lichtenstein, A. *Acta med Scandinav*, 1932, supp 49.

3 Kastlin, G. *Am J M Sc* **173** 799, 1927.

4 Cook, T J. *Am J Orthodontics* **24** 467, 580 and 687, 1938.

5 Appleton, J L T, Jr. *Dent Cosmos* **74** 267 and 371, 1932.

6 Cahn, L R. *J Am Dent A* **28** 909, 1941.

7 Epstein, C M. *Dent Digest* **38** 420, 1932.

8 Hanzlik, P J. *J Am Dent A* **22** 487, 1935.

9 Watkins, C H. *J Am Dent A* **22** 1934, 1935.

10 Brown, P K. *Am Med* **3** 649, 1902.

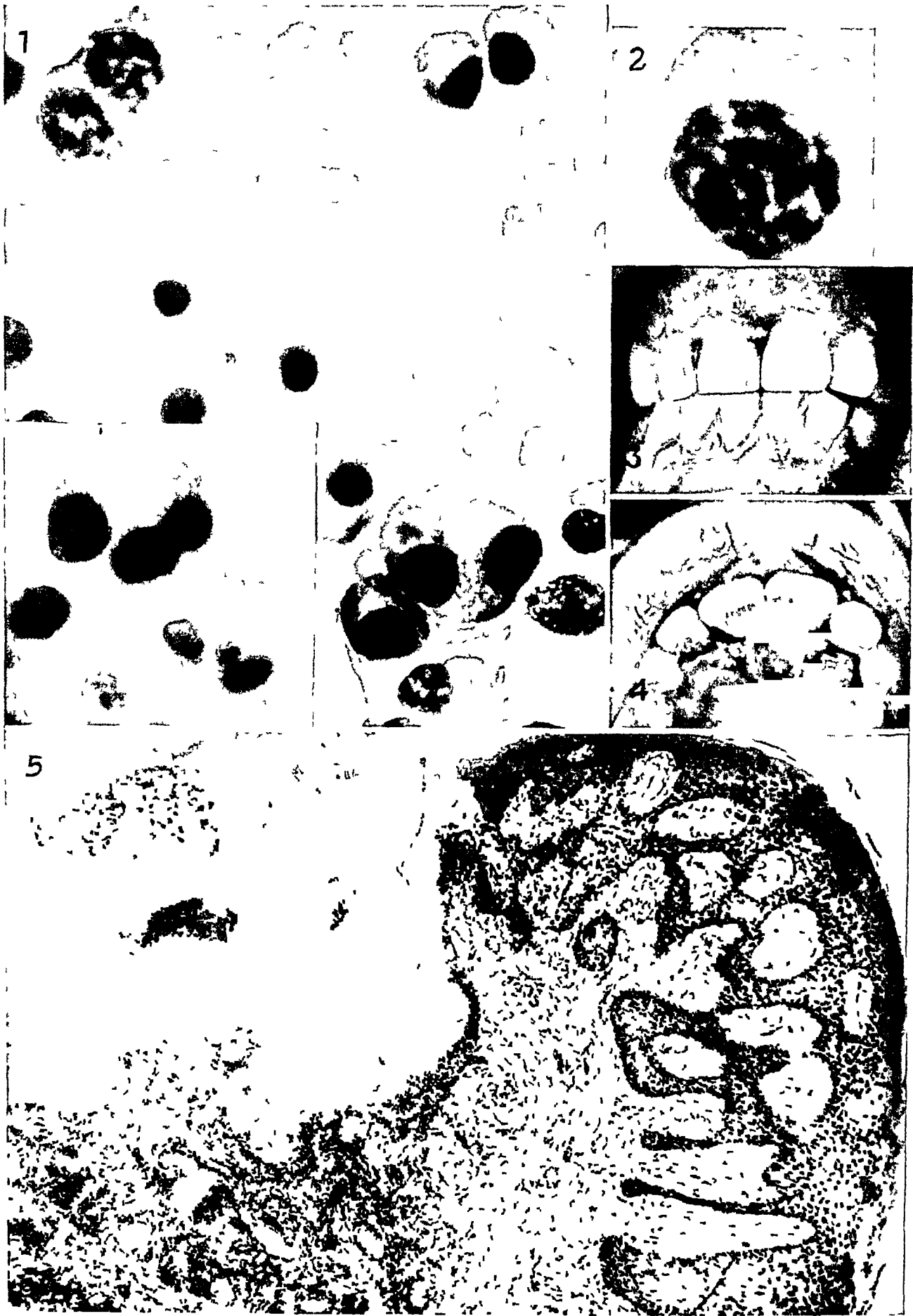
11 Lovett, B R. *J A M A* **83** 1498, 1924.

12 Rotter, W. *Virchows Arch f path Anat* **258** 17, 1925.

13 Plum, P. *Clinical and Experimental Investigations in Agranulocytosis*, London, H K Lewis & Co, Ltd, 1937.

14 Petri, E. *Deutsche med Wchnschr* **50** 1017, 1924.

15 Bernier, J L. *A Manual for the Differential Diagnosis of Oral Lesions*, St Louis, C V Mosby Company, 1942.



(See legends on opposite page)

as could be ascertained, no other biopsy or detailed study of such a gingival lesion has been reported in the medical or the dental literature

#### REPORT OF A CASE

The case is one of those that were not recognized at an early stage of the disease. A 26 year old woman was admitted to a hospital with high fever and in a critical condition. For the preceding two weeks she had been treated for "flu" and "trench mouth." She had taken some drugs for the "flu," but she could give no information as to what drugs or the amount. The first blood count was made on admission. At the same time the routine examinations were made, and for differential diagnosis a biopsy specimen of one of the smaller ulceronecrotic lesions was obtained and a sternal puncture was made. In smears from the gingival lesions, mostly fusiform bacilli and Vincent's spirochetes were seen. Blood transfusions and injections of pentnucleotide were given. However, after a brief declining course the patient died.

At admission the blood revealed moderate anemia with 2,900 white cells. The differential count showed 1 per cent eosinophils, 1 per cent basophil polymorphonuclear leukocytes, 83 per cent lymphocytes, 9 per cent monocytes and 6 per cent Turk cells. Two days later a second count showed a decrease in the white cell count to 1,050, with a complete absence of polymorphonuclear leukocytes, the differential count showed 92 per cent lymphocytes, 5 per cent monocytes and 3 per cent Turk cells. The red blood cells were normal in size and shape, and the platelets were normal in number and appearance.

Microscopic examination of the marrow (obtained by sternal puncture) revealed large numbers of plasma cells. Their basophilic cytoplasm contained a darker stained outer and a lighter stained inner zone. Vacuoles in the cytoplasm were frequent. The nucleus was eccentric and had the typical cartwheel structure. The plasma cells varied greatly in size, from 10 to 22 microns, and many were binucleated and frequently arranged in groups (fig 1), some were trinucleated (fig 1, inset).

Large phagocytic reticulum cells with an ill defined, light blue-stained cytoplasm and large, round nuclei were present, frequently in small groups (fig 1). The content of the cytoplasm of some of these reticulum cells indicated erythrophagocytosis. The plasma cells and the reticulum cells were present in the largest numbers. There were also stem cells, normoblasts, some monocytes and a few lymphocytes. No myelopoietic cells were present except some disintegrating myelocytes. The red blood cells were of normal size and shape, megakaryocytes were present in normal numbers.

The blood smears revealed Turk cells that were greatly similar to the plasma cells of marrow. They

were round, and their cytoplasm was more basophilic than that of the plasma cells. The nucleus, however, showed the typical cartwheel structure and a paranuclear lighter-stained zone (fig 2).

At the time of admission the patient had gingivitis and stomatitis. The gingiva was swollen throughout, red, with a velvety, glossy appearance. It might be compared to intestinal mucous membrane. There were necrotic areas of several gingival papillae in the lower as well as the upper jaw (fig 3). The tissues of the palate showed extensive ulceration and necrosis, especially in the region of the incisors (fig 4). The margin of the ulceration was grayish, while in the center dark areas of necrotic tissues were seen, impregnated with blood.

The patient complained of severe pain in the entire oral cavity and could swallow only with difficulty. The biopsy specimen was taken from the lower jaw, between the first and the second bicuspid on the left side. When sectioned (fig 5) it revealed extensive necrosis of the gingiva, extending through the epithelium into the connective tissue. The lesion was sharply limited toward the vital tissues. This was typical coagulation necrosis with the gross outlines of tissues still present. Some remnants of epithelium could still be seen. Bacteria were scarce. Below the necrotic area there was a narrow zone of cellular accumulation.

The epithelial surface showed no keratinization. The cells were somewhat flattened, but neither keratosis nor parakeratosis was present. In the deeper layers of the epithelium edema was manifest. The epithelial cells were separated from each other by wide spaces. The edema was most extensive in the germinative layer. The subepithelial connective tissue was very edematous, showing only a few fibroblasts, blood vessels and wandering cells. Some of the capillaries of this area were empty and contained only few lymphocytes and monocytes. In the entire specimen only one polymorphonuclear leukocyte could be observed, in a blood vessel. The loose edematous tissue contained lymphocytes, monocytes and macrophages.

The borderline between the necrotic and the living tissue is of special interest. The cellular elements in this area were mainly plasma cells and macrophages. Polymorphonuclear leukocytes were absent. Many of the plasma cells and macrophages showed signs of disintegration in this zone. Lymphocytes were scarce.

At some distance from the necrotic area but still close behind the zone of cell accumulation (fig 6) the cells were in better condition, necrosis being much less pronounced. Plasma cells and macrophages dominated the field, there were only few lymphocytes.

A striking feature of this specimen was the plasma cells. In several regions there were plasma cells with two nuclei, not only singly but in groups of two and more (fig 7). Some of the plasma cells were small, others, considerably enlarged, showing extensive anisocytosis. Several trinucleated plasma cells of giant size

#### EXPLANATION OF FIGURES 1 TO 5

Fig 1—Sternal marrow,  $\times 958$ . Mononuclear and binuclear plasma cells and reticulum cells. Plasma cells show anisocytosis. Trinucleated plasma cell,  $\times 1,917$ .

Fig 2—Blood smear showing a Turk cell,  $\times 2,396$ .

Fig 3—Ulceronecrotic lesion on the gingiva.

Fig 4—Ulceronecrotic lesion on the palate.

Fig 5—General view of a biopsy specimen of the gingiva,  $\times 43$ . Note the extensive necrosis and the narrow zone of infiltration bordering the necrotic area.



were observed (fig 8). There were mitotic figures in groups of plasma cells and also in cells within the edematous, loose subepithelial connective tissue. The size of the mitotic cells would indicate that they were plasma cells and macrophages.

In some capillaries a larger number of nongranular mononuclear cells could be seen, these were mainly lymphocytes and monocytes. In some instances these cells migrated from the capillaries into the surrounding

## COMMENT

The observations described are focused on gingivitis and necrotic ulcer.

The velvety quality, the translucency and the red color of the swollen gingiva are due to changes of the epithelium and the subepithelial connective tissue, namely, lack of keratinization in

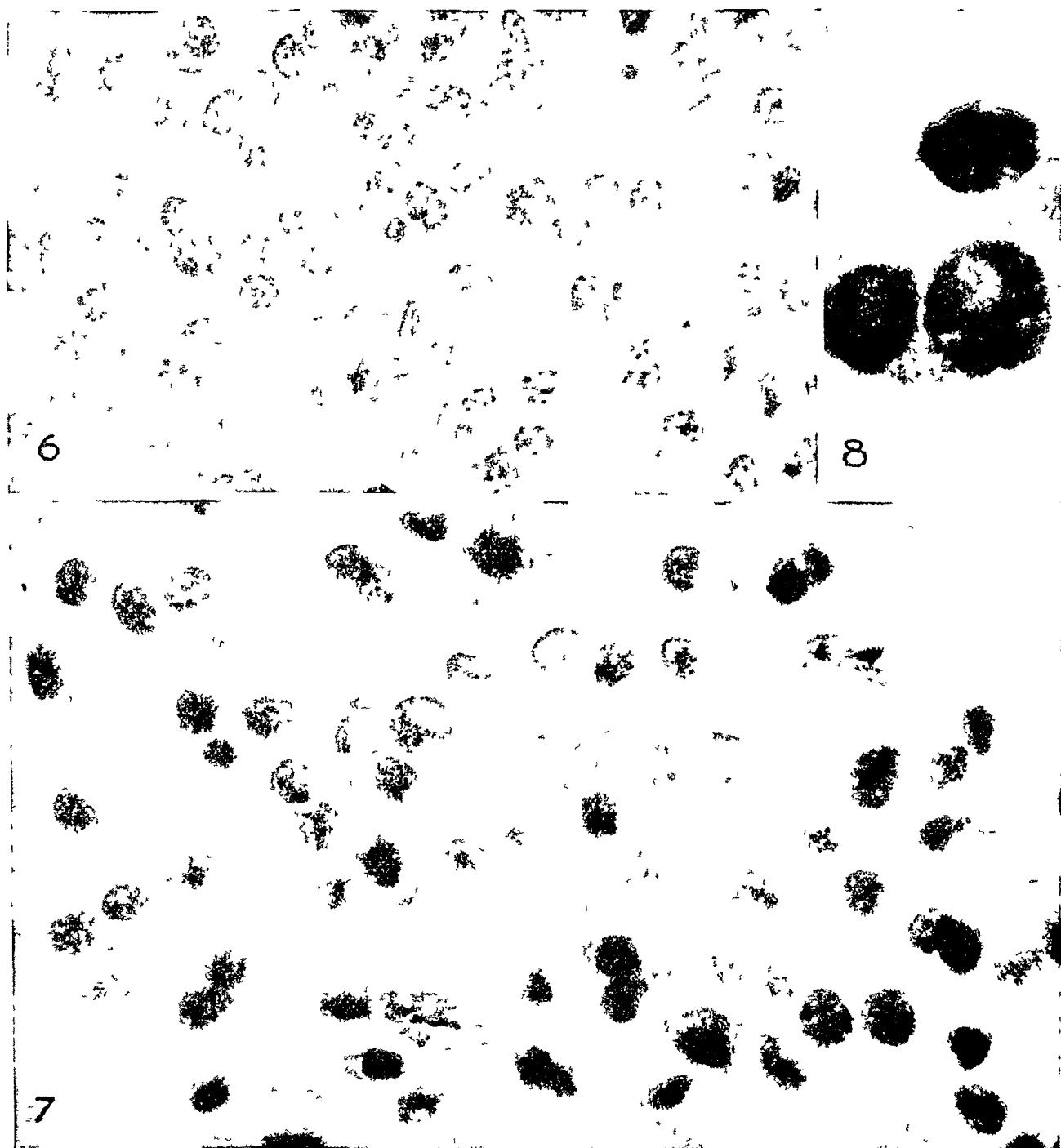


Fig 6—Tissue close to the necrotic area in the gingiva,  $\times 1,150$ . The cellular accumulation consists mainly of plasma cells and macrophages. Only few lymphocytes are present.

Fig 7—Gingiva showing several binucleated plasma cells in a group,  $\times 1,342$ . A few lymphocytes, macrophages and mononucleated plasma cells are present.

Fig 8—Trinucleated plasma cell in the gingiva,  $\times 2,300$ .

connective tissue. The endothelial lining of the capillaries revealed signs of activity. The endothelial cells were more numerous and larger than normal, i.e., swollen.

the superficial layers of the epithelium, edema of the deep layers of the epithelium, and edema and hyperemia of the subepithelial connective tissue.

The necrotic ulcer reveals necrosis lack of polymorphonuclear leukocytes, edema and infiltrating plasma cells and macrophages. The first three features are generally accepted characteristics of the necrotic lesion in agranulocytosis, they usually appear following the decrease in the neutrophil polymorphonuclear leukocytes in the blood. The closer mechanism by which they are brought about is entirely unknown.

The changes in the gingival connective tissue were characterized by a decrease in the numbers of certain cells and by an increase in those of others. The cells which decreased in number were those of vascular origin. Not only were the polymorphonuclear leukocytes practically lacking but the lymphocytes and the monocytes were negligible in number. From capillaries, crowded with large cells, only few lymphocytes and monocytes migrated into the tissues. Among the fixed tissue cells the number of fibroblasts was reduced.

Present in increased numbers were plasma cells and macrophages. The plasma cells were slightly increased in number at the zone of infiltration, they were still more numerous somewhat farther from the area of necrosis. Changes in the quality of the cells also had taken place. Binucleated cells in large numbers, frequently in groups, and trinucleated cells were likewise present. Some binucleated plasma cells and very rarely a trinucleated cell are observed in cases of common gingivitis. In the described case there was an apparent discrepancy between the moderate hyperplasia of the mononucleated and the relatively large number of multinucleated plasma cells.

The macrophages were likewise increased in number. Their cytoplasm and their nucleus were enlarged and mitotic figures could be seen.

Altogether, it is emphasized that in reaction to the necrosis the gingiva even in the absence of polymorphonuclear leukocytes exhibited proliferative changes, such as proliferation of plasma cells, macrophages and capillary endothelial cells.

In the present case the changes in the marrow showed microscopically certain similarities to the gingival changes. In the marrow the leukocyte-forming cells were lacking. Present in increased numbers were plasma cells, many of which were binucleated and some trinucleated. Plasma cells are normal inhabitants of the marrow, although to a small extent (0.2 to 1.0 per cent). In the present case they far outnumbered the other cells. The binucleated and trinucleated forms appeared numerous and in a shape similar to that seen in the gingiva. Also present

in increased numbers were reticulum cells showing erythrophagocytosis.

The type of marrow seen in different cases of agranulocytosis is not uniform. The described type is one of the most frequent and has previously been designated as "aplastic" or "empty" marrow. However, some reports refer to increased numbers of stem cells or plasma cells in such marrow patterns (Leys<sup>16</sup>, Rohr<sup>17</sup>). It is to the credit of Darling, Parker and Jackson<sup>18</sup> that emphasis was placed on the fact that this seemingly "aplastic" marrow is actually hyperplastic with respect to plasma cells, stem cells and reticulum cells, which show more or less marked proliferation.

There is, undoubtedly a striking similarity in the behavior of the plasma cells and the reticular elements between the gingiva and the marrow. In view of this similarity it is questionable whether these changes are purely local in character, confined to the inflammatory lesion of the gingiva and to the marrow. If they are of a more general character, a similar tissue reaction may be expected of other organs, wherever the plasma cells or the much more widely distributed reticulum cells are normally present. Lacking any autopsy report, we searched for observations on this probability in the literature. There are indications that in some cases plasma cells were present in more or less increased numbers in the lymph glands, the spleen, the peribronchial region of the lung (Plum<sup>13</sup>) and around necrotic lesions of the gastrointestinal tract (Lichtenstein<sup>2</sup>, Plum<sup>13</sup>). Rotter<sup>12</sup> emphasized the hyperplasia and the hypertrophy of the reticuloendothelial cells of the spleen, the lymph nodes and the liver in each of his 6 cases. Similar changes of the Kupffer cells of the liver were observed by Koch,<sup>19</sup> Hallermann,<sup>20</sup> Oppikofer<sup>21</sup> and Plum<sup>13</sup>, whereas Lichtenstein<sup>2</sup> saw no change in the Kupffer cells. The reticulum cells of the lymph nodes and the spleen showed such changes (Dahlen and Wahlgren<sup>22</sup>, Piette<sup>23</sup>, Plum<sup>13</sup>). The histologic observations of Petri<sup>14</sup> are of interest. At the examination of necrotic ulcera-

16 Leys, D. *Lancet* 2 751, 1933.

17 Rohr, K. *Folia haemat* 55 305, 1936.

18 Darling, R. C., Parker, F., and Jackson, H., Jr. *Am J Path* 12 1, 1936.

19 Koch, W. *Zentralbl f allg Path u path Anat* 48 355, 1930.

20 Hallermann, W. *Folia haemat* 42 1, 1930.

21 Oppikofer, E. *Beitr z path Anat u z allg Path* 85 165, 1930.

22 Dahlen, B., and Wahlgren, F. *Acta med Scandinav* 65 407, 1926.

23 Piette, E. C. *J A M A* 84 1415, 1925.

tions of the gastrointestinal tract she noted the same large "lymphoid-like" cells as in the marrow

It is worth while to emphasize these observations with a purpose to stimulate systematic histologic examinations on extensive and well selected material, including that from patients with different types of agranulocytosis, with different gingival lesions and marrow structures (i e, "maturation arrest" with myeloid hyperplasia) The impression was gathered that the changes observed in the gingiva and the marrow were not merely accidental or superficial They indicate, moreover, that, in the course of acute agranulocytosis, when the white cell formation is depressed, conditions are created which favor stimulation of plasma cells and reticulum cells We wish to point out the possibility that the stimulation of certain cells may not be confined to the marrow and the local necrotic lesion of the gingiva but may represent a reaction on a systemic scale It may be possible that such observations will ultimately result in an improved classification of cases of agranulocytosis

#### SUMMARY

In a case of acute agranulocytosis with ulceronecrotic gingivitis the most characteristic features were lack of keratinization of the epithelium and edema of the deeper epithelial layers, also edema and hyperemia of the subepithelial connective tissue The necrotic area was surrounded by a narrow zone of infiltration consisting of plasma cells, macrophages and a few lymphocytes Polymorphonuclear leukocytes were absent Many binucleated and some trinucleated plasma cells were noted Mitoses were present The capillary endothelial cells were swollen Migration from the capillaries was reduced to a few lymphocytes and monocytes The tissue changes around the lesion were moderately proliferative

The sternal marrow lacked myelopoietic cells but showed increased numbers of phagocytic reticulum cells and plasma cells, with many binucleated and some trinucleated forms, similar to those observed in the gingiva

The question is raised whether the plasmocytic and the reticuloendothelial proliferation in the marrow as well as in other tissues are parts of a systemic reaction

# AORTIC ABNORMALITIES IN DOGS USED FOR EXPERIMENTAL PURPOSES

W C HUEPER, M D

NEW YORK

Mainly rabbits have been used in past decades for studies on experimental arteriosclerosis and atheromatosis, and a fair amount of information has therefore become available on the occurrence of the various types of so-called spontaneous arterial lesions in this species<sup>1</sup> The increasingly frequent use of dogs for such purposes in recent years<sup>2</sup> makes it desirable that adequate data on the occurrence, the frequency, the types and the distribution of "spontaneous" arterial lesions in dogs be collected, as such information is essential

for the reliable interpretation of experimental observations on canine arteriosclerosis

It is unfortunate that the mass of the existing data on this subject deals with dogs of a relatively advanced age, i e, animals which are used comparatively rarely for experimental purposes and which for this reason are of little value when experimental results<sup>3</sup> are being analyzed The histologic examination of the aortas of 50 mongrel dogs was undertaken in an attempt to provide information on arteriosclerosis occurring in the dogs ordinarily used in experiments

From Warner Institute for Therapeutic Research  
1 (a) Bennecke, A Virchows Arch f path Anat **191** 226, 1908 (b) Dominguez, H Arch Path **5** 577, 1928 (c) Fischer, B Verhandl d Kong f inn Med **20** 235, 1905, Deutsche med Wchnschr **31** 1713, 1905 (d) Gouget, cited by von Leersum<sup>1a</sup> (e) Hedinger, E, and Loeb, O Arch f exper Path u Pharmacol **56** 314, 1907 (f) Hornowski, J Virchows Arch f path Anat **215** 280, 1914 (g) Israel, O ibid **86** 299, 1881 (h) Johnstone, cited by Lowenthal<sup>1b</sup> (i) Jores, L Beitr z path Anat u z allg Path **41** 167, 1907 (j) Kaiserling, C Klin Wchnschr **44** 29, 1907 (k) Kalamkarow, cited by von Leersum<sup>1a'</sup> (l) Kesten, H D Arch Path **20** 1, 1935 (m) Loeb, O Deutsche med Wchnschr **39** 1819, 1913 (n) Levin, I, and Larkin, J H Proc Soc Exper Biol & Med **7** 109, 1910 (o) Lowenthal, K Kreislauforgane, in Jaffe, R Anatomie und Pathologie der Spontanerkrankungen der kleinen Laboratoriumstiere, Berlin, Julius Springer, 1931 (p) Lucien, M, and Parisot, J Compt rend Soc de biol **64** 917 and 919, 1908 (q) Miles, A B J A M A **49** 1173, 1907 (r) Miller, J L J Exper Med **40** 542, 1924 (s) Onadri, cited by von Leersum<sup>1a'</sup> (t) Ostertag, B Nervensystem, in Jaffe, R Anatomie und Pathologie der Spontanerkrankungen der kleinen Laboratoriumstiere, Berlin, Julius Springer, 1931, p 514 (u) Pearce, R M J A M A **51** 1056, 1908 (v) von Rzentkowski, C Klin Wchnschr **41** 830, 1904 (w) Seegal, B C, and Seegal, D Arch Path **3** 73, 1927 (x) Seifried, O Die Krankheiten des Kaninchens mit besonderer Berücksichtigung der Infektions- und Invasionskrankheiten, ed 2, Berlin, Julius Springer, 1937, p 235 (y) Steinbiss, W Virchows Arch f path Anat **212** 152, 1913 (z) Thevenot, cited by von Leersum<sup>1a'</sup> (a') von Leersum, M Virchows Arch f path Anat **217** 452, 1914 (b') Zeek, cited by Foot, N C Am J Path **10** 705, 1934

2 (a) Dragstedt, L R, Goodpasture, W C, Vermeulen, C, and Clark, D E Am J Physiol **126** P479, 1939 (b) Dragstedt, L R, Clark, D E, Julian, O C, Vermeulen, C, and Goodpasture, W C Surgery **8** 353, 1940 (c) Hueper, W C Arch Path **27** 466, 1939, **38** 162, 1944

## EXPERIMENTAL PROCEDURE

Aortas were obtained from 50 mongrel dogs which had been killed after being subjected to acute experi-

### Age Distribution of One Hundred and Seventy-Four Mongrel Dogs

Years	1 to	2 to	3 to	4 to	5 to	6 to	7 to	8 to
Number	15	25	35	45	55	65	75	85
Per cent	46	48	40	21	10	4	1	3
	26.4	27.6	23.5	12.1	5.7	2.3	0.6	1.7

mentation carried out with the animals under pentobarbital sodium anesthesia and involving in most instances the testing of various vasoactive drugs The experiments lasted from three to eight hours and were terminated by an intravenous injection of ether The

3 (a) Fox, H Arteriosclerosis in Lower Mammals and Birds Its Relation to the Disease in Man, in Cowdry, E V Arteriosclerosis, New York, The Macmillan Company, 1933, p 152 (b) Kollisch, B Zur pathologischen Anatomie und Aetiologie der sog Atherosklerose der Arterien der Haustiere, Inaug Dissert, Berne, 1910 (c) Krause, C Beitr z path Anat u z allg Path **70** 121, 1922, Virchows Arch f path Anat **289** 352, 1933 (d) Lyding, H Ztschr f Tiermed **11** 359, 1907, Zur Kenntnis der Arteriosklerose bei Haustieren, Inaug Dissert, Giessen, 1910 (e) Otto, C Virchows Arch f path Anat **203** 352, 1911 (f) Pallaske, G Frankfurt Ztschr f Path **40** 64, 1930 (g) Spiegel, A Virchows Arch f path Anat **231** 224, 1911 (h) Stenius, P I Untersuchungen zur Kenntnis der Altersveränderungen an den Blutgefäßen des Hundes, Inaug Dissert, Leipzig, 1928 (i) Thoma, cited by Bennecke<sup>1a</sup> (j) Wolkoff, K Virchows Arch f path Anat **252** 208, 1924 (k) Zinserling, W D Beitr z path Anat u z allg Path **88** 241, 1932, **94** 20, 1934

aorta was dissected out in its entire length, leaving a small portion of the cardiac interventricular septum and the first parts of all the branches attached to the main vessel. This was then cut open lengthwise and rolled up. In this form it was carried through the usual histologic technical procedures of dehydration and

infiltration. Sections were cut from the paraffin blocks in such a way that they represented longitudinal sections through the entire aorta. The sections were stained with hematoxylin-eosin, with Hart's modification of Weigert's elastic tissue stain and with Van Gieson's stain for connective tissue.

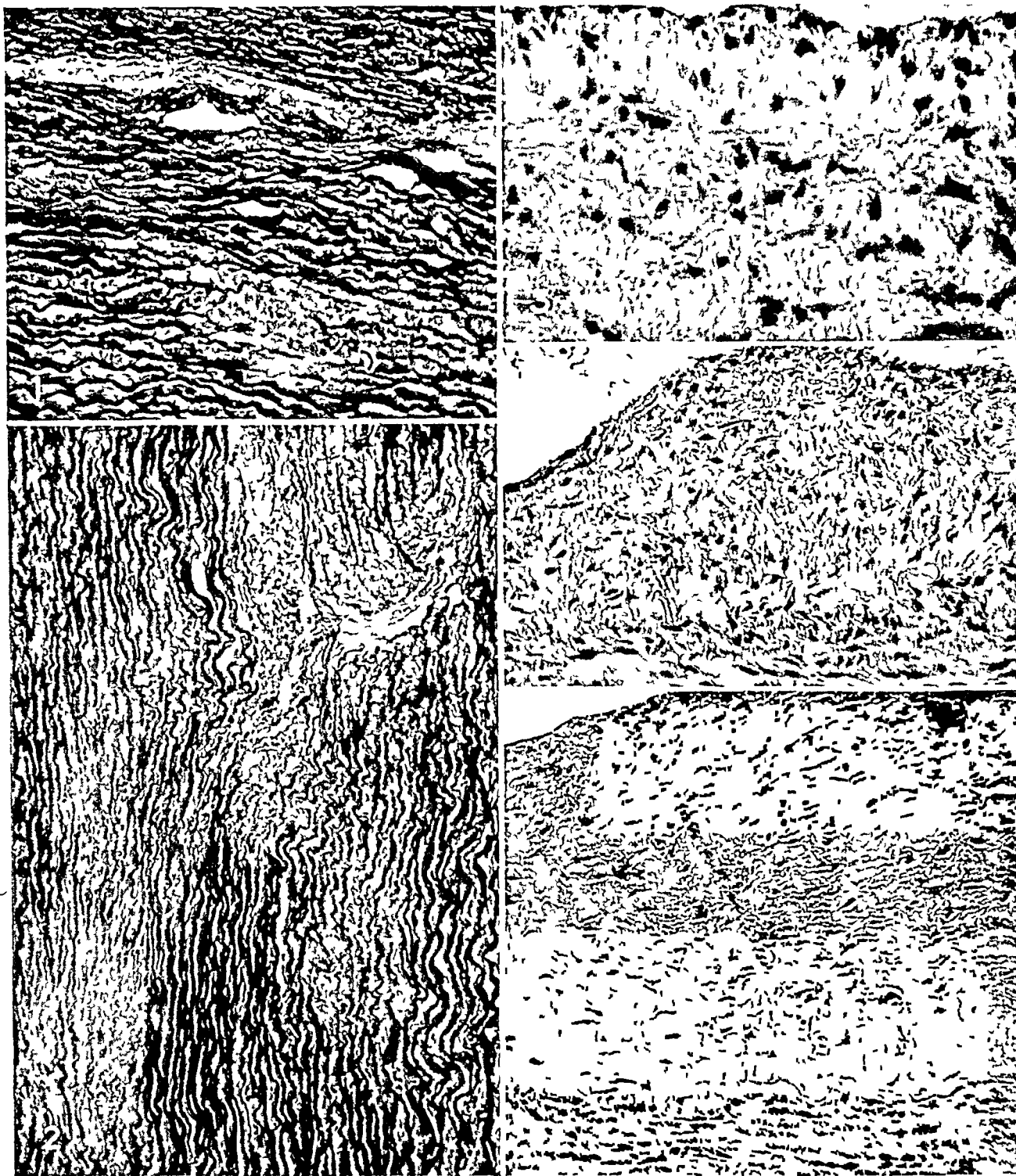


Fig 1—Crowding and fragmentation of elastic fibrils in a fibrohyaline area of the outer part of the media in the region of the aortic arch

Fig 2—Perivascular focal interruptions of the elastic membranes of the media in the ascending portion of the aorta

Fig 3—Edematous intimal thickening covering an edematous media in the ascending portion of the aorta

Fig 4—Cushion-like fibrous thickening in the abdominal portion of the aorta

Fig 5—Large fibrohyaline thickening of the lower abdominal part of the aorta

The ages of 5 of the 50 dogs were known. One was over 8 years, a second 5 years, a third 3 years and the fourth and fifth 15 years old. Inasmuch as it seemed to be important to ascertain some information concerning the approximate ages of the rest of the dogs included in this series, the ages of 174 mongrel dogs used later on in the Institute were estimated from the condition of their teeth. The age distribution shown in the table was obtained.

Ninety per cent of the dogs studied were thus in the age range of 1 to 4 years, when "spontaneous" arteriosclerosis is said to be absent in dogs.<sup>4</sup> A similar age distribution presumably prevailed among the animals whose aortas were investigated.

#### OBSERVATIONS

The histologic examination showed that the inner third of the media of the ascending portion of the aorta and often also of the proximal part of the arch was mildly, moderately or severely edematous in most dogs. The muscle bundles were separated from each other by an irregular and loose felt of fine fibrils or stringy matter staining red with Weigert's connective tissue stain. The elastic membranes in this region were usually fragmented and deranged with the exception of the innermost parts where, as a rule, a dense felt of fine elastic fibrils was present replacing there an internal elastic membrane. In the descending portions of the aorta a well developed internal elastic membrane was seen. The elastic tissue in the media was well formed, and the fibrils here were rather thick, particularly in the outer part of the media. Wherever these structures joined those of the branches a relatively sudden change in vascular structure occurred. The elastic tissue shifted almost entirely into the outer part of the media, where several thick membranes formed an external elastic coat which surrounded the inner parts of the muscular media containing scanty and delicate elastic fibrils. The muscular tissue in the branches was arranged in definite bundles, which contrasted with the homogeneous muscular coat of the aortic media.

In 6 of the 50 dogs a fibrohyaline scar was found in the outer part of the media approximately 3 to 5 cm. distal from the aortic base. The cicatricial tissue contained usually a moderate number of capillaries and occasionally a few lymphocytes. Collagenous material predominated in such lesions and surrounded fragmented, interrupted, straightened and partly condensed, partly atrophic elastic fibrils (fig. 1). The location of this lesion in the region of the arch corresponded to that of the ligamentum arteriosum. In a moderate number of aortas the presence of cir-

cumscribed elastic tissue defects apparently located around or near vasa vasorum was noticed in the media (fig. 2). These interruptions in the elastic membranes appeared in the specially stained sections as "blanched" areas. They affected particularly the central and outer portions of the media. It may be finally mentioned that in 2 dogs there was seen at the distal rim of the aortic bulb large cartilaginous foci surrounded by hyaline tissue forming nodes which projected from the intimal-medial border zone into the vascular lumen.

In addition to these structural variations of nonarteriosclerotic and in part physiologic or congenital nature, the following lesions of doubtful or definite arteriosclerotic significance were observed. Small loose edematous intimal thickenings were seen in the ascending portion of the aorta in 3 dogs (fig. 3). They contained a stringy material staining red with Van Gieson's method and occasionally a few monocytes. A local crowding of endothelial cells in such areas was present in 2 of these dogs. In 2 additional dogs small fibrous intimal thickenings were found in the same location close to the base of the aorta. In one of the two lesions numerous muscular elements were embedded in an intertwining collagenous matrix. A network of delicate elastic fibrils was present in the intimal cushions in both dogs. A small hyaline intimal thickening was seen in the thoracic portion of the aorta of a sixth dog, and several large fibroblastic or fibrohyaline cushions were observed in the abdominal portion of the aorta of a seventh dog (figs. 4 and 5).

A small area of fragmentation and calcification of the internal elastic membrane was noted in the ascending portion of the aorta of an eighth dog (fig. 6), while a ninth dog exhibited in the media of the ascending portion of the aorta several small scattered areas in which the muscle cells had a homogeneous, pink-stained cytoplasm and were loosely packed, indicating localized muscular degeneration and atrophy (fig. 7). The cellular nuclei were distinctly diminished in number.

The proximal parts of the numerous aortic branches, especially the common iliac arteries, which were often included in the sections were normal with the following exception. The medial sacral artery of the oldest dog of this series revealed a considerably and concentrically thickened fibrohyaline intima and a calcified internal elastic membrane causing a distinct narrowing of the vascular lumen (fig. 8). The thickening was free from elastic fibrils.

<sup>4</sup> (a) Strauch, C. Beitr. z. path. Anat. u. z. allg. Path. 61: 532, 1916. (b) Krause.<sup>2c</sup>

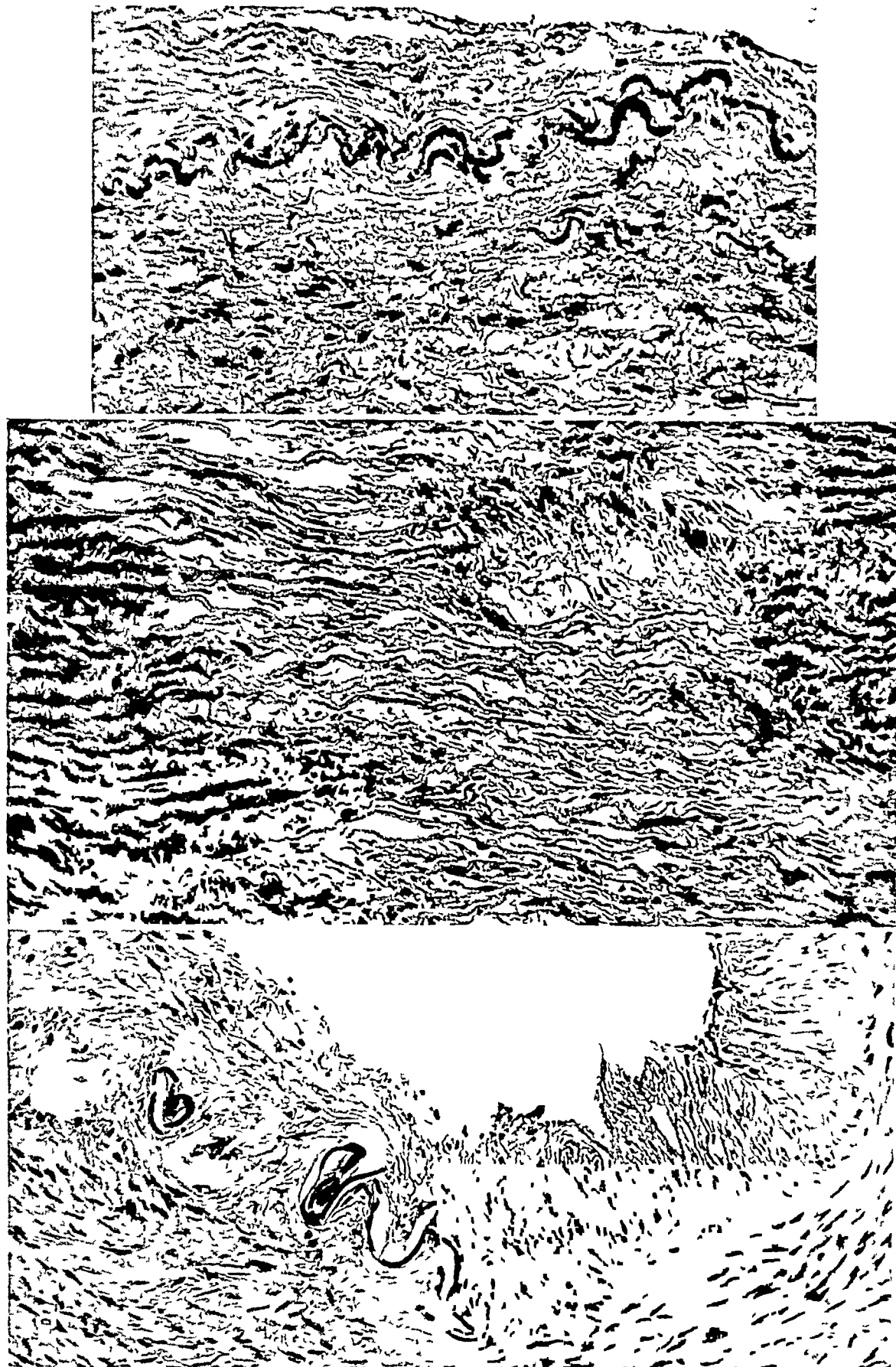


Fig 6—Calcification and fragmentation of the internal elastic membrane of the ascending portion of the aorta  
Fig 7—Circumscribed degeneration of the media of the aorta  
Fig 8—Concentric fibrous intimal thickening with calcification of the internal elastic membrane of the medial sacral artery



## COMMENT

Marked edema of the inner part of the media of the ascending portion of the aorta, present in the majority of the dogs, was reported by Zinserling<sup>3a</sup> as a normal condition of the canine aorta. It has been looked on as an agonal phenomenon related to prolonged circulatory failure preceding the final arrest of circulation. This concept was supported by Wiesel<sup>5</sup> and by Segre and Kellner,<sup>6</sup> who made similar observations in the arteries of man and animals. Segre and Kellner considered the albuminous matter filling the interstices between the widely separated muscle bundles as the product of agonal contraction of the muscle cells by which cellular fluid is expressed into the intercellular spaces. This interpretation, however, is in disagreement with that placed on this matter by Sternberg<sup>7</sup> and Albrecht.<sup>8</sup> These investigators regarded the edematous imbibition of the arterial media as a post-mortem change. Wiesel,<sup>5</sup> on the other hand, thought that it accompanied exclusively acute or chronic cardiac and infectious diseases associated with circulatory failure. The loose edematous structure of the ascending portion of the aorta of the dog is not a consistent phenomenon, as it was absent in some 5 aortas.

The presence of cartilaginous tissue in the distal rim of the aortic bulb observed in 2 dogs is probably related to the occurrence of similar cartilaginous or, occasionally also, osseous plates in the aortic rim in various animals, including dogs (Spiegel<sup>3g</sup>, Hueper<sup>2c</sup>), and does not seem to represent a part of an arteriosclerotic process.

The occurrence of circumscribed defects in the elastic tissue such as those observed in some of the aortas has been previously recorded by Krause and Strauch, who found such tears in the elastic membranes of even young dogs.<sup>4</sup>

Of the edematous or fibrosing intimal thickenings present in the ascending portion of the aorta in 5 dogs, those of edematous type were obviously of relatively acute and reversible nature and all of them were comparatively small and circumscribed and thus possibly early arteriosclerotic developments. Only the fibrohyaline intimal cushions found in the abdominal portion of the aorta of 1 dog can be considered definite and advanced arteriosclerotic lesions, which were associated with granular calcification of the fragmented and swollen internal elastic mem-

brane present underneath some of these reactions. Thus in only 1 dog of a total of 50 was well developed, though localized, arteriosclerosis of the aorta demonstrable. Inasmuch as the 2 dogs, 8 years and 5 years old, respectively, showed merely small intimal thickenings of the ascending portions of the aorta, it can be assumed that the animal with arteriosclerosis must have been either especially well advanced in age, because the great majority of the dogs were doubtlessly not more than 4 years old, or represents an exceptional occurrence of arteriosclerosis in a young dog.

These observations demonstrate that young dogs can be used for the experimental production of arteriosclerosis without fear of any appreciable and significant spontaneous arteriosclerotic lesions being encountered. They do not lend support to the claim that the distemper usually developing in young dogs has any etiologic role in the development of such changes as contended by Kollisch<sup>3b</sup> and denied by Strauch.<sup>4a</sup> The anatomic type of lesions observed is that noted after severe and prolonged fluctuations in the tonus of vessels or in intravascular hydrostatic pressure. It is purely speculative, however, whether or not the marked changes in blood pressure readily elicited in dogs by psychic stimuli have any causal relation to the sclerosing arterial manifestations frequently seen in old dogs. Fox stated that domesticated dogs show arteriosclerosis after the fifth year of life as pebbly granular thickenings in the lower thoracic and abdominal portions of the aorta, consisting of fibrous intimal hyperplasia rarely breaking through the internal elastic membrane, with occasional calcifications of the media underneath. He noted that aortic calcifications occur in domestic dogs rarely and are then usually found in the beginning portion of the aorta.

Kollisch,<sup>3b</sup> who studied 50 working dogs, 2 to 7 years old, and 50 luxury dogs, 8 to 20 years old, recorded arteriosclerosis in 10 of the working dogs and in 3 of the luxury dogs, or in 23 per cent of the total number. The lesions of 7 of these dogs consisted of partly rough-surfaced, partly smooth-surfaced round or irregular intimal thickenings, while in the other 16 dogs round projecting intimal cushions existed. In 2 instances the entire aorta was calcified. The intimal thickenings were of the fibrous type, containing fat only rarely. Medial lesions exhibited local fibrosis and occasionally calcification, with fibrillar cartilage sometimes surrounding the calcified foci. Strauch<sup>4a</sup> made a thorough study of the various large and medium-sized arteries of 56 dogs, 47 of them aged 1 to 5, and 9 of them aged 5 to 8.

<sup>5</sup> Wiesel, J. *Wien Arch f inn Med* **1** 197, 1920.

<sup>6</sup> Segre, R., and Kellner, E. *Centralbl f allg Path u path Anat* **32** 561, 1921-1922.

<sup>7</sup> Sternberg, C. *Wien klin Wchnschr* **31** 297 and 310, 1921.

<sup>8</sup> Albrecht, H., cited by Segre and Kellner.<sup>6</sup>

He examined the following arteries the aorta, the innominate, subclavian, common and external carotid, celiac, superior mesenteric, phrenic, renal, internal spermatic, femoral, hypogastric, medial sacral, coronary, splenic, hepatic and pulmonary arteries and in some cases also the cerebral, brachial and saphenous arteries. In only 1 of the 47 young dogs early arteriosclerotic changes were seen (2.2 per cent). Cushion or ridgelike rough intimal fibrous thickenings of the aorta, on the other hand, were noted in 6 of the 9 old dogs (67 per cent). The lesions were usually located distal from the entrances of the renal arteries. Fat was rare and scanty in these foci. The observations as to the comparative frequency of arteriosclerosis in old dogs made by Strauch<sup>41</sup> are supported by the findings of Zinserling<sup>31</sup> in 26 old dogs (8 to 28 years of age). Every one of these old dogs had fibrous intimal thickenings of the aorta, all except 3 had lipid deposits in the aorta and all of them had such lesions in the splenic artery. The fat was usually found in the form of fine droplets or as a diffuse imbibition in the ground substance of the media of the ascending part of the aorta, often arranged along the elastic membranes. Macrophages with fat were occasionally seen. The type of fatty infiltration of the canine aorta thus differs distinctly from that found in human atherosclerosis. Extensive medial calcinosis and fibrosis, occasionally associated with the appearance of cartilaginous tissue, was observed by Zinserling<sup>31</sup> in the aortas of old dogs (11 to 16 years). Krause,<sup>30</sup> on the other hand, missed any medial calcification in even the oldest dog studied by him, and noted

medial calcifications of the aorta in only 2 dogs with generalized arterial calcifications, which he attributed to a disturbance of the calcium metabolism. Fibrous intimal thickenings of the aorta are, according to him, commonly present in dogs more than 5 years old.

Special mention may be made of the circular fibrous intimal thickening of the medial sacral artery of 1 dog. In view of the fact that this was practically the only aortic branch which revealed any sclerotic change and that the sclerosis was of concentric type involving the entire circumference of the vessel, consideration must be given to the possibility that changes in the normal hydrostatic pressure may have been responsible for this lesion. It is the medial sacral artery which supplies blood to the tail of the dog. An amputation of this organ would result in a temporary marked increase of intravascular hydrostatic pressure which conceivably may elicit the reported type of arterial change. This explanation however, must remain speculative, as no data are available on the question whether or not this particular dog had an amputated tail.

#### SUMMARY

Arteriosclerosis of the aorta is exceptional in dogs before the age of 5. The arterial lesions met with in dogs are predominantly of the sclerosing type. The highly edematous state of the inner part of the media of the ascending portion of the aorta frequently seen in dogs is either a normal anatomic condition or an agonal phenomenon resulting from circulatory disturbances.

# COMBINED EFFECTS OF AN ESTROGEN AND AN ANTERIOR HYPOPHYSIAL EXTRACT ON THE SKELETON OF THE GROWING MOUSE

MARTIN SILBERBERG, M D, AND RUTH SILBERBERG, M D

ST LOUIS

Estrogen and a hormone produced by the anterior lobe of the hypophysis accelerate skeletal aging in growing animals<sup>1</sup> However, the two hormones accomplish this effect in different ways The anterior hypophysial hormone stimulates growth and hastens epiphysiodiaphysial union by inducing premature regression and resorption of the epiphysial cartilage, estrogen, on the other hand, inhibits growth and resorption, thus delaying epiphysiodiaphysial union In view of these contrasting mechanisms we thought it of interest to investigate the combined effects of these two hormones on skeletal development and aging

## MATERIAL AND METHODS

Thirty-two virgin female mice 5 to 6 weeks old were used Of these, six pairs of D mice received subcutaneous injections of 100 rat units of alpha estradiol benzoate in sesame oil<sup>2</sup> once weekly and intraperitoneal injections of 0.1 cc of an extract of the anterior lobe of the bovine hypophysis five times weekly for one week, one, three, five, ten and sixteen months, respectively, seven pairs of C<sub>3</sub>H mice were treated correspondingly for four, six, seven, eight, nine, ten and eleven months, three pairs of A mice received the same treatment for one week, two weeks and one month The anterior hypophysial extract was prepared according to the method of Loeb and Basset<sup>3</sup> Untreated mice and animals which had been treated only with the estrogen or with the anterior hypophysial extract and which had been used in former experiments, served as controls The mice were kept on a standard diet of Purina dog chow with water available at all times At the end of the experimental period they were killed with chloroform, the lower part of a femur and the upper part of a tibia

This investigation was aided by the David May-Florence G May Fund

From the Laboratory of Research Pathology, Washington University School of Medicine, the Snodgrass Laboratory, City Hospital, and the Laboratory of the Jewish Hospital

1 Silberberg, M, and Silberberg, R Arch Path 36 512, 1943

2 The alpha estradiol benzoate in sesame oil was supplied by Dr Erwin Schwenk, of the Schering Corporation

3 Loeb, L, and Basset, R B Proc Soc Exper Biol & Med 26 860, 1929

were taken out as a whole and prepared for histologic examination<sup>4</sup>

## HISTOLOGIC EXAMINATION

The following description is based on the observations of the tibia

*Epiphysial Disk*—After one week of the combined treatment the epiphysial cartilage proliferated by way of mitosis and underwent marked hypertrophy However, these processes were less intensified than after injections of the anterior hypophysial extract alone The individual cartilage cell row was composed of eight columnar and one or two hypertrophic cells The corresponding figures for untreated animals were seven or eight columnar and two or three hypertrophic cells For mice receiving the anterior hypophysial extract alone the figures were seven or eight columnar and one or two hypertrophic cells In animals given the estrogen only, there were but five or six columnar and one or two hypertrophic cells in the individual cartilage row, the mitotic proliferation and the conversion of columnar into hypertrophic cartilage cells were markedly decreased On the whole, after one week's injections of the estrogen and the anterior hypophysial extract the growth zones resembled closely those seen in mice treated with the anterior hypophysial extract alone, but calcification and ossification were more accentuated than in either the latter or in normal animals

If the two hormonal preparations were allowed to act for two weeks, the epiphysial plate was narrower than ordinarily Mitotic proliferation and hypertrophy of the columnar cartilage were still marked There were five or six columnar and one or two hypertrophic cells instead of seven columnar and two or three hypertrophic cells as is normal In animals given the anterior hypophysial extract only, six or seven columnar and two or three hypertrophic cells were counted in the single cell row, in mice receiving the estrogen alone the corresponding figures were five or six columnar and one or two hypertrophic cells Therefore, under the combined influence of both hormones the width of the epiphysial plate was comparable to that found after injections of the estrogen alone, however, both proliferation and hypertrophy of the epiphysial cartilage were less decreased than after administration of the estrogen alone

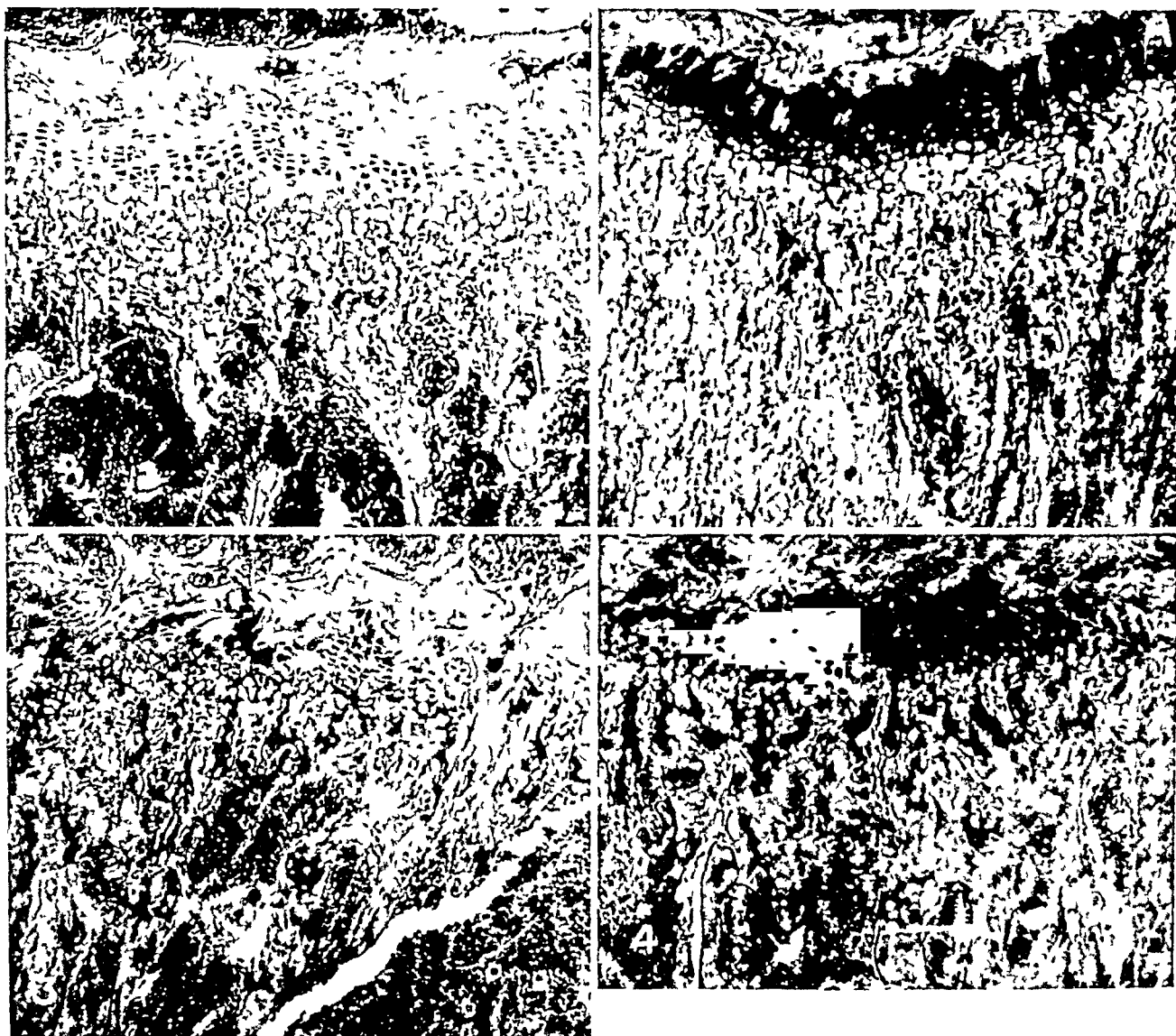
After one month of the combined treatment the narrowed growth zones were densely calcified In

4 (a) Silberberg, M Proc Soc Exper Biol & Med 32 1423, 1935 (b) Silberberg, M, and Silberberg, R Am J Anat 68 69, 1941

areas where calcification was somewhat lighter there were four or five columnar cartilage cells and one hypertrophic cell in a single row. Both cell types were smaller than usual, mitoses in the cartilage were scarce, calcification was more prominent than after administration of the anterior hypophyseal extract alone. The growth zone appeared at this stage not unlike that seen after injections of the estrogen alone, in animals treated only with the anterior hypophyseal extract bony plugs had replaced the destroyed cartilage cell rows and were already being resorbed by advancing marrow (figs 1 to 4).

zones which showed resorption by capillaries advancing from the marrow.

After four or five months of combined treatment the resorption was even more accentuated. In mice of strain C<sub>3</sub>H the epiphyseal plate had to a large extent been replaced by a fibro-osteoid tissue originating in the metaphysis. In nontreated animals, thick bony plugs were present in the inactive cartilage, but there were no perforations of the growth zones. Under the influence of the anterior hypophyseal extract alone, perforations of the epiphyseal disks appeared at this stage but without the formation of fibro-osteoid tissue. In



Each of the sections shown was made through the epiphyseal disk of the upper part of a tibia of a virgin mouse of strain A 9 weeks old.

Fig 1—Section from an untreated animal,  $\times 100$ . The growth zone shows regular configuration.

Fig 2—Section from a mouse given 100 rat units of an estrogen by injection once a week for one month,  $\times 100$ . The growth zone is narrowed and heavily calcified, interlacing trabecular bone is seen in the metaphysis.

Fig 3—Section from a mouse given 0.1 cc of a bovine anterior hypophyseal extract by injection five times weekly for one month,  $\times 100$ . The epiphyseal cartilage is broken down, showing plug formation.

Fig 4—Section from a mouse given both the estrogen and the anterior hypophyseal extract for one month,  $\times 100$ . Note the narrowed, densely calcified growth zone and the numerous thick mature trabeculae in the metaphysis.

Administration of both preparations for three months further increased calcification and ossification of the cartilage. Numerous bony plugs traversed the growth

mice receiving only the estrogen the growth zones still consisted of a continuous layer of calcified cartilage, bone deposition in the epiphyseal plate was predominant,

whereas resorptive processes were insignificant (figs 5 to 8)

If both hormonal preparations were given for from six to nine months, the fibro-osteoid tissue was resorbed and replaced by cellular marrow. The wide gaps in the epiphyseal plate were even more conspicuous, since untreated mice of strain C<sub>3</sub>H show little tendency toward perforations of the growth zones. Thus the skeletal age of the epiphyseal disk was comparable to that of untreated virgin mice during the second year of life. After administration of the estrogen alone, no such perforations were found, in mice receiving only the anterior hypophyseal extract, resorption progressed, still without production of fibro-osteoid tissue.

If the combined treatment was continued further, only scanty remnants of calcified cartilage were left of the epiphyseal disk or it was converted into a thin bony scar, both rather uncommon occurrences in old untreated mice. After fourteen months of injections, epiphyseodiaphyseal union was not so far advanced as in animals given the anterior hypophyseal extract alone, however, resorptive processes were more advanced than in mice receiving only the estrogen (figs 9 to 12).

*Diaphysis*—After one and two weeks of the combined treatment the osteoblasts in the metaphysis and along the bony trabeculae proliferated by way of mitosis. There was more connective tissue in the subepiphyseal layer than usual, the spicules were thicker than ordinarily or after treatment with either hormonal preparation alone. There was less vascularization than after injections of the anterior hypophyseal extract but more than after administration of the estrogen only. The trabeculae were firmer, shorter and more widely spaced than under the influence of the estrogen.

After one month of the combined treatment a transverse bony plate had been laid down beneath the calcified cartilage, indicating cessation of growth. The metaphyseal connective tissue had further increased in density and amount, osteoblastic proliferation showed a marked decline but no standstill as in estrogen-treated animals, the trabeculae were coarser, more numerous and much thicker than during the earlier stages. However, the interlacing bony network, so prominent after injections of estrogen alone, was not found (figs 1 to 4). The vessels of the shaft were moderately filled, mitotic proliferation of the periosteum and the endosteum was scarce or lacking, the compacta was thicker than usual and contained numerous small osteocytes. In untreated animals osteoblastic proliferation was still marked at this age. After injections of the anterior hypophyseal extract, osteoblastic proliferation was stimulated, the shafts contained enlarged capillaries, and the periosteum and the endosteum were loosened. In estrogen-treated animals, on the other hand, the vascular canals were narrowed and less numerous than ordinarily, a dense connective tissue had developed in close approximation to the thickened shafts, and mitotic proliferation in this tissue was scarce, if present at all.

After three months of treatment the transverse bony plate underneath the calcified cartilage was irregular and thinned out. It was connected with the interlacing spicules now present throughout the metaphysis and in the corners between the metaphyseal plate and the shaft.

This network was at the same time being resorbed by a fibro-osteoid tissue which also penetrated into the remnants of the epiphyseal disk (figs 5 to 8). Between the spicules and the fibro-osteoid tissue some marrow was found. Farther distally, the hemopoietic marrow was more prominent, but it still contained undissolved irregular trabeculae. This or a similar histologic pattern was never observed in the metaphysis of any untreated mouse or of any receiving only the anterior hypophyseal extract. The condition resembled that seen in mice treated with the estrogen for six or more months, that is, twice as long as in the series given the combined treatment.

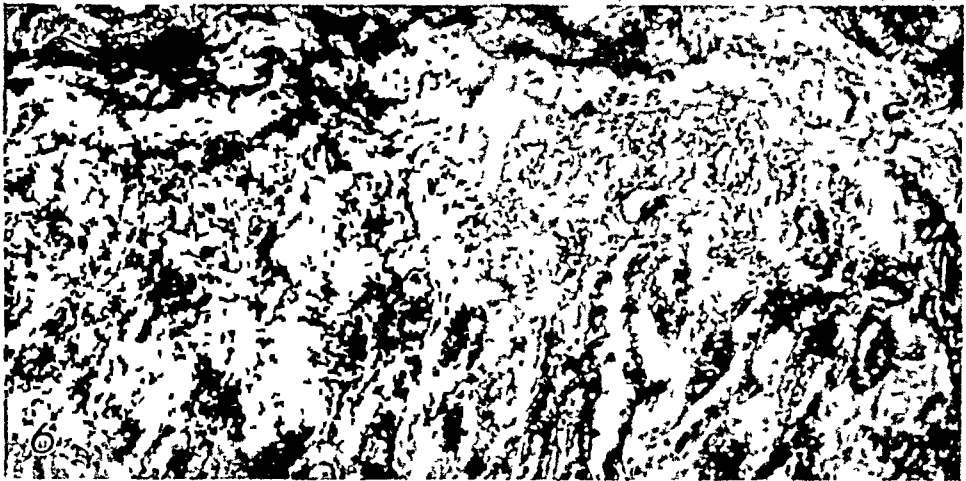
After four, five or six months of the combined treatment the resorptive processes were accentuated. The bony plate underneath the calcified cartilage was corroded from below by marrow, the connective tissue in the metaphysis showed better vascularization and less hyalinization than at the earlier stages, the spicules were thinned out and shortened, and the shafts were likewise thinner. There was less bone present than before and less than after injections of the estrogen alone. However, bony tissue was more abundant than in either untreated animals or mice receiving the anterior hypophyseal extract.

If the administration of both the estrogen and the anterior hypophyseal extract was continued, the resorptive processes were further intensified, and they were ahead of those found after treatment with the estrogen alone. In mice treated for seven to nine months, there were only traces of a thin bony network in the marrow cavity, and after sixteen months most of the excess bone and the connective tissue had disappeared. There were still scanty remnants of irregular spicules which distinguished this series from untreated animals as well as from those given the anterior hypophyseal extract alone (figs 9 to 12).

*Joints*—In mice treated with both the estrogen and the anterior hypophyseal extract for from one week to one month the articular cartilage cells underwent slight to moderate hyperplasia and hypertrophy and at the same time some degeneration, changes that were classified as grade I. With increasing duration of the experiment, these alterations did not make much progress. As a rule, the cartilaginous matrix underwent pronounced hyalinization. Only 1 animal showed articular changes of grade II, namely, moderate proliferation and regression of the cartilage. Grade III, that is typical osteoarthritic changes, were not observed in the animals given the combined treatment, whereas such lesions did occur in many untreated old mice.<sup>5</sup> Administration of the estrogen alone decreased the incidence and the severity of these articular changes, conversely, treatment with the anterior hypophyseal extract alone hastened their onset and increased their incidence and severity.<sup>6</sup> Under the influence of both preparations the hyalinization of the cartilage caused by the estrogen probably counteracted the liquefying effect of the growth-stimulating factor in the anterior hypophyseal extract and thus prevented a breakdown of the articular cartilage.

<sup>5</sup> Silberberg, M., and Silberberg, R. *Anat Rec* **91** 89, 1945

<sup>6</sup> Silberberg, M., and Silberberg, R. *Am J Path* **17** 189, 1941



Each of the sections shown was made through the epiphysial disk of the upper part of a tibia of a virgin mouse of strain C<sub>3</sub>H 6 months old

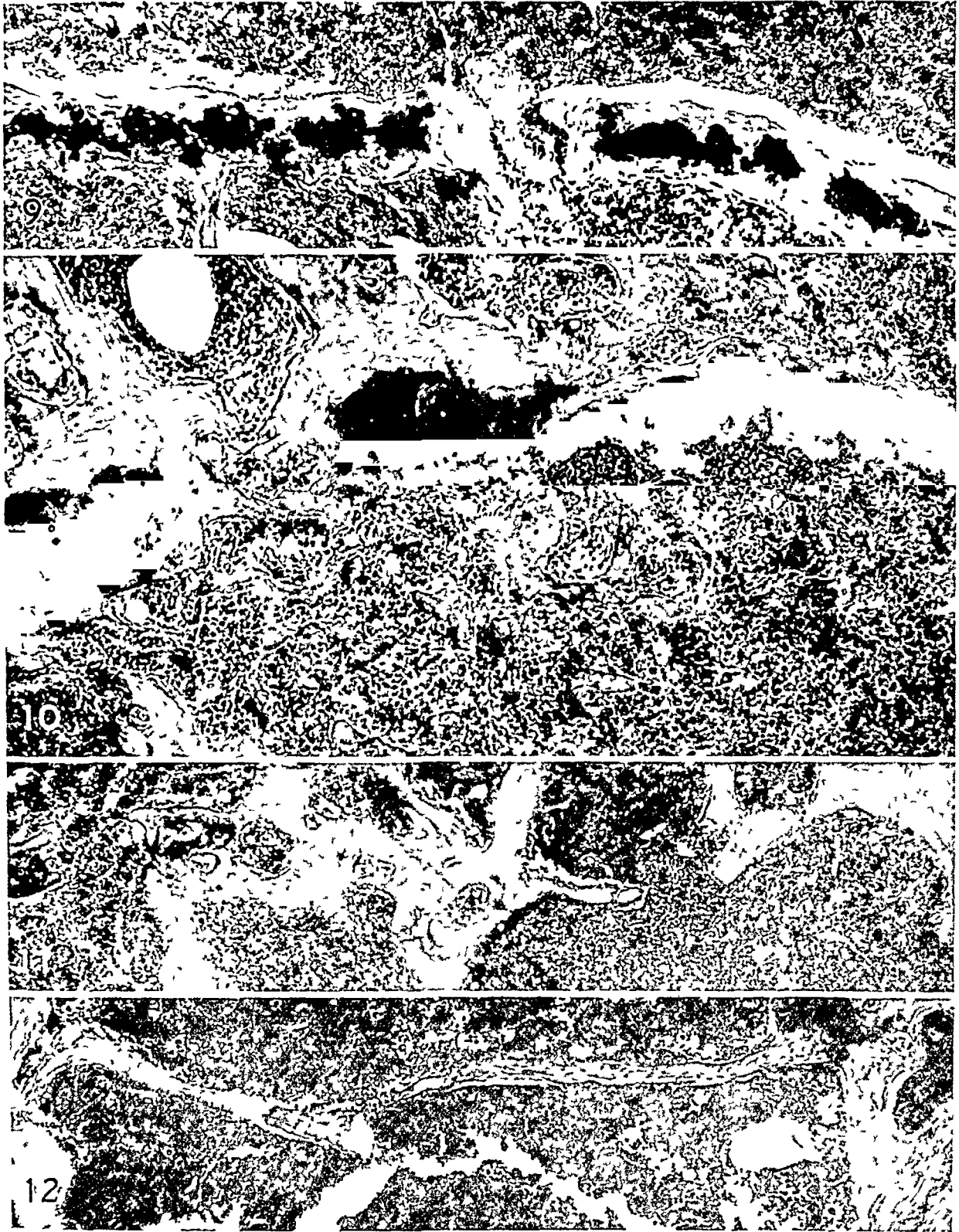
Fig 5—From an untreated animal,  $\times 100$  Note plugs of regressed cartilage in the inactive growth zone

Fig 6—From a mouse which had received 100 rat units of an estrogen by injection once a week for five months,  $\times 100$  A dense interlacing trabecular network may be seen in the metaphysis, with scanty remnants of marrow between the spicules

Fig 7—From a mouse which had received 0.1 cc of a bovine anterior hypophysial extract by injection five times weekly for five months,  $\times 100$  The growth zone is represented by a bony plate containing remnants of calcified cartilage Note the wide communication between the epiphysis and the diaphysis

Fig 8—From a mouse to which both the estrogen and the anterior hypophysial extract had been administered for five months,  $\times 140$  In the left upper corner of the growth zone are small amounts of cartilage Fibro-osteoid tissue has developed in the epiphysis and the diaphysis





Each of the sections shown was made through the epiphysal disk of the upper part of a tibia of a virgin mouse of strain D 17 months old  $\times 100$

Fig 9—Section from an untreated animal,  $\times 100$  Note remnants of calcified cartilage in the growth zone perforated by bone marrow

Fig 10—Section from a mouse given 100 rat units of an estrogen by injection once weekly for sixteen months,  $\times 140$  Note remnants of calcified cartilage in the growth zone and formation of fibro-osteoid tissue in the metaphysis

Fig 11—Section from a mouse given 0.1 cc of a bovine anterior hypophyseal extract by injection five times weekly for sixteen months,  $\times 100$  There is complete epiphysiodiaphyseal union, with wide communications between the epiphysis and the diaphysis

Fig 12—Section from a mouse to which both the estrogen and the anterior hypophyseal extract had been administered for sixteen months,  $\times 100$  A bony scar has taken the place of the growth zone The marrow is cellular, and no trabecular bone is present



COMMENT

A certain antagonism between the effects of anterior hypophysial and estrogenic hormones has been demonstrated in various tissues. An extract of the anterior lobe of the hypophysis loosens the connective tissue<sup>7</sup> and increases the vascularity and the uptake of water by the tis-

TABLE 1—Skeletal Changes After One Month of Treatment

		Anterior Hypophysial Extract	Estrogen	Anterior Hypophysial Extract and Estrogen
Epiphysial cartilage	Growth	Increased	Decreased	Somewhat decreased
	Regression	Increased	Increased	Markedly increased
Metaphysis	Connective tissue	Loosened, with vascularization increased	Hyalinized, with vascularization decreased	Somewhat hyalinized, with vascularization decreased
	Bone	Ossification increased, short trabeculae	Resorption decreased, interlacing trabeculae	Fairly long, mature thick trabeculae

sues.<sup>8</sup> Estrogen, on the other hand, promotes the deposition of hyalin in the uterus, the vagina and the adrenal glands,<sup>9</sup> in cartilage and in marrow,<sup>10</sup> and it decreases the water content of the long bones.<sup>11</sup>

In view of these antagonisms it might be assumed that estrogen and the growth-promoting hormone of the anterior lobe of the hypophysis would each diminish the effect of the other. However, this does not seem to be generally true. The accompanying tables demonstrate in a schematic way skeletal changes caused by injections of (a) an anterior hypophysial extract, (b) estradiol benzoate in sesame oil and (c) both together.

After one month of the combined treatment (table 1), the growth of cartilage was less decreased, and the connective tissue was less hyalinized and better vascularized, than after injections of the estrogen alone. Moreover, the increased resorption caused by the anterior hypophysial extract prevented the interlacing of the bony

spicules seen after treatment with the estrogen. Whereas these findings may be interpreted as antagonistic effects, there was also a certain synergism of the two hormones noticeable. The regression of the cartilage was more marked, and the bone was thicker, than after treatment with either alone. The changes in the bone represent a combination effect involving two different mechanisms. The anterior hypophysial extract stimulated osteoblastic bone formation, the simultaneously injected estrogen diminished the osteoblastic activity. But this decrease in active bone formation was more than compensated for by the lag of resorption of the primary spicules.

The findings after six months of treatment are summarized in table 2. The synergistic action of the estrogen and the anterior hypophysial extract is reflected in the marked resorption of the epiphysial plate, which was more advanced than after treatment with either alone. Whereas under the influence of the anterior hypophysial hormone the epiphysial plate was resorbed by marrow, in the series given both preparations it was dissolved by fibro-osteoid tissue. The latter appeared earlier than in animals treated with the estrogen alone. In no instance was this tissue tumorous in character; it disappeared as soon as the excessive bone had been resorbed. This suggests that the fibrous metaplasia of the marrow represents a reaction of the organism to the excess bone and not simply a specific hormonal effect. In normal animals, as well as in those treated with the an-

TABLE 2—Skeletal Changes After Six Months of Treatment

	Anterior Hypophysial Extract	Estrogen	Anterior Hypophysial Extract and Estrogen
Epiphysial cartilage	Inactive, with marked resorption by marrow	Ossifying, inactive, with little resorption	Marked resorption by connective tissue, fibro-osteoid tissue present
Metaphysis	Hemopoietic marrow present, few thin trabeculae	Much trabecular bone, some interlacing	Fibro-osteoid tissue present
Articular cartilage	Softened, hypertrophic and hyperplastic	Hyalinized	Hyalinized

terior hypophysial extract, the solution of the trabeculae is accomplished by capillaries or body fluids. The more abundant and the denser the bone, the more inadequate are the normal mechanisms of solution. It is conceivable that the additional resorptive power of connective tissue is needed to restore the normal tissue equilibrium. A direct stimulation of the connective tissue by

7 Loeb, L., in Harvey Lectures, Baltimore, Williams & Wilkins Company, 1940, vol. 36, p. 228.  
8 Downs, W. G., Jr. Arch. Path. 12: 37, 1931.  
Long, C. N. H., in Luck, J. M. Annual Review of Physiology, Stanford University, Calif., Annual Reviews, Inc., 1942, vol. 4, p. 465.  
Silberberg and Silberberg.<sup>1</sup>  
9 Loeb, L. The Biological Basis of Individuality, Springfield, Ill., Charles C. Thomas, Publisher, 1945.  
10 Silberberg, M., and Silberberg, R. (a) Arch. Path. 28: 340, 1939, (b) Am. J. Anat. 69: 295, 1941.  
Landauer, W., and Zondek, B. Am. J. Path. 20: 179, 1944.  
11 Lippman, H. N., and Saunders, J. B. de C. M. J. Endocrinol. 3: 370, 1944.

the anterior hypophysial hormone may partly account for the early fibrous metaplasia of the marrow

In a previous investigation<sup>7</sup> we examined the effects of syngenesiotransplants of the anterior lobe of the mouse hypophysis. Not only did these transplants give off hormones acting on the skeleton but they also stimulated the production of estrogen in the transplant-bearing host. The latter was thus exposed to increased amounts of both anterior hypophysial and estrogenic hormones. In these animals the growth of cartilage was inhibited presumably because of the predominance of the estrogen effect. On the other hand, the anterior hypophysial hormone apparently maintained the proliferation of the cartilage longer than ordinarily. Whereas these findings likewise indicated an antagonistic action of the two hormones, others suggested a certain synergism. As in the present series, the regression of the epiphysial cartilage was more intense and more bone was formed than after treatment with either the estrogen or the anterior hypophysial extract alone.

Thus, the skeletal effects of anterior hypophysial and estrogenic hormones are opposed to each other in some respects, but they do not neutralize each other. In the present experiments the estrogen effect on the bone tended to outbalance that of the anterior hypophysial hormone. The following factors may be responsible for this occurrence. 1. The anterior hypophysial extract loses its effectiveness with prolonged administration.<sup>1</sup> 2. There is a gradation in the reaction of various species to hormonal stimulation. The sensitivity of the skeletal tissues to estrogen increases in the following order: guinea pig, rat, mouse; on the other hand, mice respond to anterior hypophysial extract less readily than guinea pigs. The predominance of the estrogen effect in our mice may thus have been due to the greater responsiveness of this species to estrogen than to the anterior hypophysial hormone. 3. The dose of the estrogen given may have been too high in proportion to that of the anterior hypo-

physial extract. However, it is difficult to obtain consistent bone changes in mice with doses less than 100 rat units weekly. On the other hand, the dose of the anterior hypophysial extract given was fairly high already.

The present observations suggest that estrogen acts on the skeleton directly and not by mediation of the anterior lobe of the hypophysis. If the estrogen effects were the result of an inhibition of the anterior lobe of the hypophysis, they should have been counteracted by the administration of an extract of the anterior lobe of the pituitary gland. Moreover, if the estrogen effects were mediated in some other way by the anterior lobe of the hypophysis, they should be lacking in hypophysectomized animals. However, in pigeons hypophysectomy did not measurably modify the medullary bone formation and the increase of the serum calcium seen under the influence of estrogen.<sup>12</sup> Furthermore, in hypophysectomized mice estrogen increased the ash content of the long bones, although to a lesser extent than in intact animals.<sup>13</sup>

#### SUMMARY

In growing mice the skeletal effects of estrogen can be modified by the simultaneous administration of an extract of the anterior lobe of the hypophysis. The two hormones oppose each other in their action on the growth of cartilage, but they cooperate in accelerating the age changes in the latter and in the overproduction of bone, anterior hypophysial hormone by stimulating osteoblastic bone formation and estrogen by promoting hyalinization of the marrow and by inhibiting the resorption of bone. Thus, the two hormones do not neutralize each other but each tends to exert its own effects on the receptor tissue. The skeletal effects of estrogen are direct ones and not mediated by the anterior lobe of the hypophysis.

12 Riddle, O., Rauch, V. M., and Smith, G. C. *Endocrinology* **36**: 41, 1945.

13 Gardner, W. U., and Clouet, D. H. *Anat. Rec.* **88**: 433, 1944.

# Case Reports

## TULAREMIA

### A Report of a Laboratory Infection Fatal on the Fifth Day, with Early Pulmonary Involvement, Autopsy

L. L. ASHBURN, M.D., Surgeon, United States Public Health Service  
BETHESDA, MD

AND

SEWARD E. MILLER, M.D., Surgeon, United States Public Health Service  
BALTIMORE

Tularemia in man has been well characterized from the standpoint of pathology in various reports based on autopsies in 73 cases. In the great majority of these cases the interval between the onset of the disease and death exceeded twelve days, and we have found only 1 case reported with autopsy in which the duration of the disease was less than eight days (four days seven hours).<sup>1</sup> We wish to report a case with autopsy in which the duration was four days sixteen hours. In this case cough was one of the earliest symptoms, *Pasteurella tularensis* was present in the sputum sixteen hours after onset, and the only organisms demonstrated in the sections of the pneumonic lung were morphologically consistent with *P. tularensis*.

#### REPORT OF A CASE

R. P., a white woman aged 55 years, was admitted to the United States Marine Hospital, Baltimore, complaining of cough, fever, headache, weakness, and pains in bones, muscles and joints. Except for Q fever in 1940, the past medical history was of no interest.

The patient was a technician in a laboratory where tularemia and leptospirosis were under experimental investigation. However, she did not come in intimate contact with tularemic animals and did not handle cultures of *P. tularensis*.

On Sept. 6, 1944, about 5 p. m., while on her way home from work, the patient felt feverish and shortly afterward a severe headache, cough, nausea and pains in muscles, bones and joints developed, with general malaise. The cough was irritating and dry. She slept poorly that night and had two or three loose bowel movements. In the morning her temperature was 103 F., and the symptoms were more severe, particularly the cough. Blood and sputum were obtained for culture and animal inoculation, and she was taken to the hospital at 4 p. m., September 7. On arrival her temperature was 104.4 F., with a pulse rate of 120.

Examination on admission showed moderate obesity, flushing of the face, moist skin, slightly inflamed conjunctivas with increased lacrimation, a somewhat congested nasal mucous membrane with little seromucoid discharge, a moderately inflamed and slightly edematous throat and some coating of the tongue. The lymph nodes were neither enlarged nor tender. Dry crackling rales were heard throughout both lung fields. Respiration was normal. There were no areas of dullness. The remainder of the examination showed only varicosities of the legs and hyperactive reflexes.

From the Pathology Laboratory, National Institute of Health, Bethesda, Md., and the Department of Pathology, United States Marine Hospital, Baltimore.

<sup>1</sup> Simpson, W. M. Arch. Path. 6:553, 1928.

A roentgenogram of the chest made twenty-four hours after onset of the illness showed in the hilar portion of the right lung an early infiltrating lesion. Supportive and symptomatic treatment was instituted, but the patient's condition gradually became worse. A second roentgenogram, made sixty-six hours after onset, showed dense infiltration radiating outward from the right hilus. September 9 administration of 1 Gm. of sulfadiazine every four hours was begun. September 10 the patient's condition was still worse with a temperature of 105.2 F., a pulse rate of 104 and respirations 64; the administration of sulfadiazine was discontinued, morphine given and the patient placed in an oxygen tent. September 10 the animals inoculated with the patient's sputum, collected sixteen hours after onset of the disease, were reported as suggesting tularemia, so that evening the patient received two 500 cc. transfusions of whole blood from immune donors and 60 cc. of Foshay's antitularemic serum in 1,000 cc. of 5 per cent dextrose in saline solution. During the night, however, she became more restless, more cyanotic and irrational. The blood pressure dropped from 145 systolic to 120 systolic and 110 diastolic. Early in the morning of September 11 respiration became quite rapid and weak, cyanosis deepened, and death occurred at 9:05 a. m., approximately four and one-half days after onset of illness.

On the morning of September 8 the blood gave a red cell count of 4,454,000, a hemoglobin content of 13.5 Gm. and a white cell count of 12,500. The differential count showed 80 neutrophils, of which 18 were stab forms, 19 lymphocytes and 1 a monocyte. Toxic granules were present in the neutrophils. The sedimentation rate was 51 mm. in one hour. Examination of sputum (smear and culture) showed a predominance of *Streptococcus viridans*; pneumococci were not found. Serum agglutination reactions for typhoid H, paratyphoid A, and paratyphoid B bacilli were positive to a dilution of 1:20. Culture of the blood, examination of the stool, dark field examination of the urine and serum agglutination tests with typhoid O, *Brucella abortus*, *Proteus* OX-19, *P. tularensis*, *Leptospira canicola* and *Leptospira icterohaemorrhagiae* as antigens gave negative results. Cultures of stool and urine were negative for pathogenic bacteria. The urine showed albumin (+++), 4 to 6 pus cells per high power field, many granular casts and an occasional red blood cell.

**Autopsy.**—External examination revealed nothing of significance. Each pleural space contained 30 to 40 cc. of yellowish fluid, and 20 cc. was present in the pericardial sac.

The left lung was soft and crepitant, and weighed 580 Gm. The cut surface showed congestion and edema throughout. The right lung weighed 820 Gm. There were marked edema and congestion of the upper and middle lobes. The upper portion of the lower lobe was completely consolidated, and over this portion there were

numerous petechial hemorrhages. On section of the lobe there was seen in the consolidated portion an oval area, measuring 3 by 5 cm, which appeared granular, more dense and of lighter color than the surrounding tissue. The hilar lymph nodes on the right side were moderately enlarged and anthracotic.

The aorta showed a little atherosclerosis.

The peritoneal cavity contained 140 cc of yellowish fluid. The liver weighed 1,900 Gm, it had a rather light color, and the cut surface showed yellowish mottling but no focal necrosis. The spleen weighed 220 Gm, it had a soft pulp but showed no foci of necrosis.

With the exception of a small shallow, apparently inactive ulcer in the duodenum, the gastrointestinal tract was normal. The gallbladder showed considerable edematous thickening. The adrenal glands were congested, with a few small hemorrhages. The kidneys were not enlarged, the cut surfaces were congested and there was some loss of demarcation between cortex and the medulla. The urinary bladder contained 350 cc of urine. The pancreas was congested. A 7 mm fibroid was present in the left cornu of the atrophic uterus. The heart, the ureters, the ovaries and the fallopian tubes were normal.

**Microscopic Examination—Lung** In the completely consolidated areas an apparent nodularity was seen under low power, produced by variation in the character of the exudate. Undulating and anastomosing bands in which the alveoli were filled with a largely fibrinous exudate separated areas in which the alveolar exudate was almost entirely cellular. This microscopic pseudonodular effect was heightened by the fairly sharp line between the alveoli with cellular and those with largely fibrinous exudate. The cellular exudate in some blocks showed marked karyorrhectic necrosis with loss of identity of the cells. Caseous necrosis of the exudate was not present, and necrosis of alveolar septums was seen in only a few small areas. In areas of less advanced karyorrhexis many cells were identified as large mononuclears, the nuclei being round, indented or lobated. Also in such areas occasional to few polymorphonuclear leukocytes were seen. While fibrin was generally not present in these alveoli, a few showed thick and often fragmented fibrin strands. In the alveoli in which the copious fine-meshed fibrin formed the bulk of the exudate, cells were present usually in small numbers. These cells usually were well preserved and could be identified with certainty. Polymorphonuclear leukocytes and large mononuclear cells were present in about equal number, sometimes the one, sometimes the other type, predominating. The alveoli bordering the area of complete consolidation contained lesser amounts of fibrinocellular, serocellular or serous exudate. Here also the cells were well preserved and moderate numbers of polymorphonuclear leukocytes were identified. The somewhat thickened and edematous alveolar septums focally were infiltrated by a few lymphocytes and large mononuclears and were often covered by a continuous layer of hypertrophic cuboidal cells. In consolidated areas the small bronchi and bronchioles were filled with exudate similar to that in the alveoli, fibrin usually being abundant. The walls showed a loose structure, some edema and patchy infiltration by lymphocytes, mononuclears and fewer polymorphonuclear leukocytes. In some the epithelium was absent from a portion of the wall, with the exudate lying directly on the basement membrane, necrotic epithelial cells were rare. The perivascular connective tissue, the interlobular septums and the pleura showed changes similar to those of the bronchial walls, with considerable variation in degree in different areas. The pleura was covered by a layer of laminated fibrin in which relatively few cells were present.

Two blocks of tissue from the grossly nonconsolidated portions of the lung showed small areas in which changes in the alveolar septums were similar to those at the border of consolidated areas, though less marked. In such areas the alveoli contained a little serum, fibrin or few to many cells. Although some of these were macrophages, polymorphonuclears usually were the most numerous.

**Bronchial Lymph Nodes (right)** These nodes showed a few focal lesions, the largest of which measured 2 mm in diameter. Each lesion was formed of large mononuclear cells showing advanced karyorrhectic necrosis in the central part of the lesion. The lymphoid follicles were small, and there was moderate irregular reticulo-endotheliosis of sinus and pulp. Many of the reticulum cells contained particles of carbon. Bacteria were not found.

**Heart** A moderate number of muscle fibers showed epinuclear accumulation of brown granular pigment.

**Liver** The central half to three fourths of all lobules of the liver showed narrowing of liver cell cords, corresponding widening of sinusoids, oxyphilia of the cytoplasm of liver cells, variable degrees of karyolysis, absence of some cells and frank necrosis of a few. In such areas there were a few to a moderate number of neutrophils between cell cords and sinusoids and in the sinusoids. In periportal areas the liver cells contained a few fine fat droplets. This graded to the center of lobules, where the fat was moderate in amount, here a few fat globules were large.

**Kidney** A little oxyphilic coagulum was present in some glomerular spaces. The epithelial cells of convoluted tubules were large and granular. A very few small patches of interstitial cortical fibrosis were seen.

**Spleen** The spleen showed moderate congestion of pulp and sinus with very slight irregular polymorphonuclear leukocyte infiltration. There was one lesion present in the pulp. It measured 500 microns in diameter and was similar to those in the lymph nodes. The follicles were small and showed no activity. Bacteria were not found.

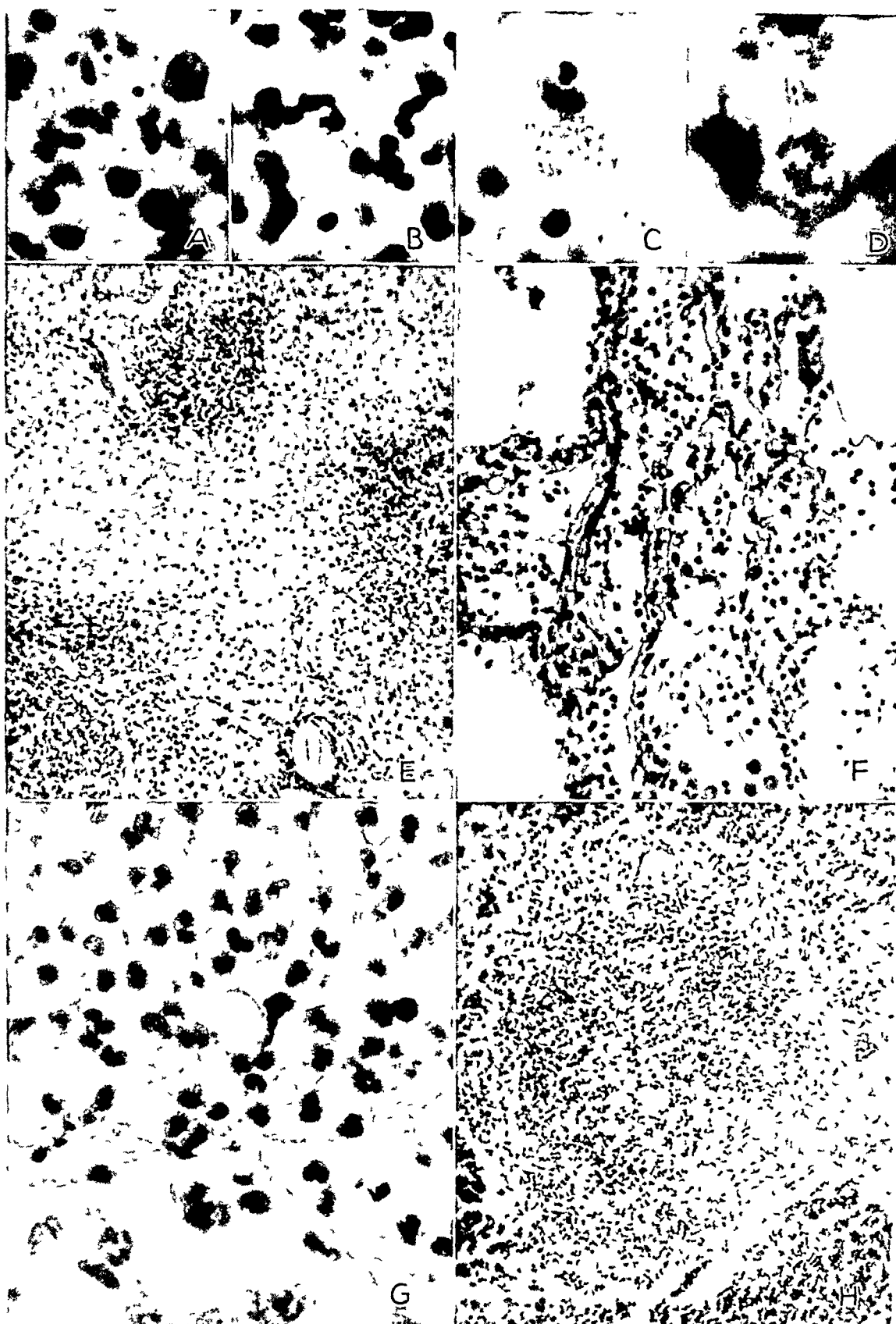
**Stomach and Duodenum** There was slight focal lymphocyte infiltration in the deep mucosa of the pylorus and among Brunner's glands of the duodenum.

**Adrenal Glands** The adrenal glands showed slight focal interstitial lymphocyte infiltration of the cortex. In one adrenal gland there was a 15 mm centrally located adenoma formed of spongiocytes.

**Gallbladder** The perimuscular coat showed marked edematous thickening with a few lymphocytes in the inner part of this layer.

**Uterus** The endometrium was atrophic. A small leiomyoma was present in the outer myometrium. There was considerable fibrosis of the wall of the cervix.

**Demonstration of Bacteria by Staining** All of the tissues for histologic study were fixed in a 3.8 per cent solution of formaldehyde. In paraffin sections of the lungs stained with hematoxylin and azure eosinate,<sup>2</sup> very faintly stained small intracellular bodies were seen. For their further study the buffer level of the stain mixture was shifted from pH 4.6 to pH 5. This gave a preparation that was too blue for good nuclear detail, but the basophilia of the minute intracellular bodies was considerably increased. In such a preparation the bacteria were seen as minute coccoid and coccobacillary forms and were gram negative. They were found in greater numbers in the cellular exudate showing only slight karyorrhectic necrosis. They were, however, seen frequently in the areas marginating the completely consolidated lung and in this location were found with greater ease since the exudate was well preserved. They



*A, B, C and D, intracellular coccoid and coccobacillary organisms in exudate of pulmonary alveoli. A, B and C,  $\times 1,000$ , D,  $1,500$ . E, consolidated lung showing contrasting cellular and fibrinous exudate,  $\times 100$ . The scattered cells enmeshed in fibrin are relatively well preserved. F, small area of recent pulmonary involvement,  $\times 200$ . Many such areas were present in the grossly nonconsolidated portion of the lung. G, alveolar exudate in an area similar to that shown in F,  $\times 775$ . The majority of the cells are polymorphonuclear leukocytes. H, focal lesion in bronchial lymph node,  $\times 90$ .*

occurred in both mononuclear cells and polymorphonuclear leukocytes, usually in small clusters. A few cells filled with the organisms were seen in scattered areas. Their distribution was patchy, and when one parasitized cell was found, others in the same alveolus also were likely to contain bacteria. They were found in cells of the bronchial exudate but were not seen in the swollen cells lining the alveoli. In identifying these organisms as *P. tularensis*, minute pigment granules, nuclear fragments and mast cell granules were seen and recognized as such. No other bacteria were observed.

*Cultural Studies and Animal Inoculations*—This part of the investigation was made by Passed Assistant Surgeon Carl L. Larson. The following paragraphs are from his report:

"Specimens of sputum and blood were obtained on September 7, about sixteen hours after the onset of illness. Cultures of whole blood on dextrose-cystine-blood agar remained sterile, and mice inoculated with blood failed to show signs of illness. No cultures were made of the sample of sputum, but the specimen was emulsified in salt solution and injected subcutaneously into the guinea pigs and intraperitoneally into mice. These animals were ill on September 9 and cultures of *P. tularensis* were obtained from both guinea pigs and mice.

"Material taken at the autopsy was not cultured on mediums as it was thought that the tissues were contaminated. All were emulsified separately in salt solution and inoculated into guinea pigs. All these animals became ill between the third and the fifth day after inoculation and cultures of *P. tularensis* were obtained from animals inoculated with cardiac blood, liver, spleen and two specimens of lung tissue. No other material was examined."

#### COMMENT

The exact number of cases of death from tularemia in which autopsies have been made is difficult to determine since a few were included among collected cases as unpublished data and later were reported in detail by different authors. Including collected cases and original reports, Lillie and Francis<sup>3</sup> in 1936 and Foshay<sup>4</sup> in 1937 published pertinent data on 51 cases. Since that time we have found published reports of 22 cases in which necropsy was done,<sup>5</sup> making a total of 73. In all of these cases except that of Simpson<sup>1</sup> the duration of the disease was eight days or longer. In Simpson's case the duration was four days seven hours.

3 Lillie, R. D., and Francis, E., in Lillie, R. D., and others. *The Pathology of Tularemia*, National Institute of Health Bulletin 167, United States Treasury Department, Public Health Service, 1936.

4 Foshay, L. *Arch Int Med* **60** 22, 1937.

5 (a) Pund, E. R., and Hatcher, M. B. *Ann Int Med* **10** 1390, 1937. (b) Mathews, W. R. *New Orleans M & S J* **90** 479, 1938. (c) Kimmelstiel, P., and Caldwell, H. W. *Am J Path* **15** 127, 1939. (d) Kitzmiller, K. V. *Ann Int Med* **12** 1375, 1939. (e) Ziferstein, I. *J Iowa M Soc* **30** 65, 1940. (f) Dredge, T. E. *M Bull Vet Admin* **16** 337, 1940. (g) Blackford, S. D., and Casey, C. J. *Arch Int Med* **67** 43, 1941. (h) Ransmeier, J. C., and Schaub, I. G. *ibid* **68** 747, 1941. (i) Anschuetz, R. R. *Am J Dis Child* **62** 150, 1941. (j) Kennedy, J. A. *J A M A* **118** 781, 1942. (k) Thomas, H. B. *Ann Int Med* **17** 659, 1942. (l) David, J. K., Jr., and Owens, J. N., Jr. *Am J Dis Child* **67** 44, 1944.

Certain evidence in this case suggests that infection took place by the upper respiratory route. The patient, a laboratory technician, did not handle the infected animals or cultures of the organisms. Cough and fever were among the first symptoms, and *P. tularensis* was demonstrated by animal inoculation in the sputum obtained sixteen hours after the onset of illness. At this time similar inoculation of blood gave negative results, and culture of the blood obtained forty hours after onset also gave negative results. It appears also that significance should be attached to the fact that only one lesion was found in the sections of spleen, whereas multiple and larger lesions were seen in the bronchial lymph nodes. Comparison of roentgenograms revealed pulmonary involvement twenty-four hours after the onset of illness. In our opinion, the circumstances and findings just related strongly suggest that pulmonary involvement was primary, with septicemia and involvement of bronchial lymph nodes and spleen secondary. It is realized that accidental ingestion of contaminated material must be considered in this case, but we find no evidence to support such a thesis.

The extent of the pulmonary involvement was much greater than that represented by the consolidated area seen on gross section. In paraffin sections made from the nonconsolidated portions, we noted some edema fluid, fibrin and variable numbers of cells in many alveoli, with associated swelling of the alveolar epithelium. Such involvement evidently contributed to the production of the cyanosis noted clinically. The microscopic observations on the lungs of this patient varied from those on the lungs of most patients whose cases have been published previously in showing more fibrin, absence of caseous necrosis and more polymorphonuclear leukocytes in the exudate. It is likely that the two latter variations are related to the short duration of the disease in that polymorphonuclears appeared to be more frequent and exudate much better preserved at the advancing margins of the pneumonic focus. It has been pointed out by some authors that karyorrhectic mononuclear cells may be mistaken for polymorphonuclears. This is indeed true, but our reference to these cells is based on their identification in well preserved exudate. The statement is often made that the presence of polymorphonuclears in significant numbers indicates a secondary infection with pyogenic bacteria. While this may be the explanation in many cases, the only bacteria found in the lung in this case were morphologically similar to *P. tularensis*. It is possible that early in the exudative process polymorphonuclears are fairly numerous and only later are overshadowed by the mononuclear reaction.

*P. tularensis* has been identified rarely in stained human tissues although prolonged search has been made for it with use of special staining procedures. Lillie and Francis<sup>3</sup> were unable to identify the organisms in their material.

They had the opportunity to study tissue from some of their collected cases in which the finding of *P. tularensis* had been reported. They also were unsuccessful in identifying them in these tissues and pointed out that confusing basophilic bodies may occur in degenerating tissue, and that Gram stains should be made to exclude the gram-positive cocci. In the 22 cases with autopsy<sup>5</sup> that we have found recorded since 1937, bacteria morphologically similar to *P. tularensis* were described in tissue sections in 1 case<sup>5b</sup> and in tissue smears (made at autopsy) in 3 others<sup>5k</sup>. We feel certain of the microscopic identification of the organisms in the lung of our patient, based on morphology and their occurrence in clusters in certain cells and supported by the fact that the organisms were recovered from sputum collected sixteen hours after the onset of illness and from lung tissue after death (animal inoculation).<sup>6</sup>

6 Drs R. D. Lillie and Edward Francis examined the stained sections of lung in this case and agreed that the organisms were morphologically consistent with those seen in experimental animals.

The route of infection, the apparently primary pulmonary involvement, the short duration and the apparent lack of resistance on the part of the patient are probably related to the facility with which the organisms were demonstrated in the lungs.

#### SUMMARY

In the case of laboratory tularemia with autopsy reported the evidence suggests that infection took place by the upper respiratory route. *P. tularensis* was demonstrated in the consolidated lung. No other organisms were found. A few necrotic foci were observed in the bronchial lymph nodes and one in the spleen. There was centrilobular degeneration of the liver but no focal lesions. *P. tularensis* was isolated by animal inoculation from the sputum sixteen hours after onset and from the lung, the liver, the spleen and the cardiac blood at autopsy. Inoculation of animals with blood and culture of blood on the first day of illness and on the second day gave negative results.

---

## Books Received

---

STUDIES OF BURNS AND SCALDS (REPORTS OF THE BURNS UNIT, ROYAL INFIRMARY, GLASGOW, 1942-1943). By L. Colebrook, T. Gibson, J. P. Todd, A. M. Clark, A. Brown and A. B. Anderson. Medical Research Council, Special Report Series no. 249. Price \$1.20. Pp. 210, with 50 illustrations. London: His Majesty's Stationery Office and New York: British Information Service, 1944.

MASS MINIATURE RADIOGRAPHY OF CIVILIANS FOR THE DETECTION OF PULMONARY TUBERCULOSIS (GUIDE TO ADMINISTRATION AND TECHNIQUE WITH A MOBILE APPARATUS USING 35 MM. FILM AND RESULTS OF A SURVEY). By Kathleen C. Clark, P. D'Arcy Hart, Peter Kerley and Brian C. Thompson. Medical Research Council, Special Report Series no. 251. Pp. 135, with 51 illustrations. Price 90 cents. London: His

Majesty's Stationery Office (New York: British Information Service) 1945.

TRAUMA IN INTERNAL DISEASES WITH CONSIDERATION OF EXPERIMENTAL PATHOLOGY AND MEDICOLEGAL ASPECTS. By Rudolph A. Stern, M.D., assistant attending physician, City Hospital, New York. Pp. 575. Price \$6.75. New York: Grune & Stratton, Inc., 1945.

A SYMPOSIUM ON MAMMARY TUMORS IN MICE. PUBLICATION OF THE AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE No. 22. By Members of the Staff of the National Cancer Institute, National Institute of Health, United States Public Health Service. Edited by Forest R. Moulton. Pp. 223, illustrated. Washington, D. C.: American Association for the Advancement of Science, 1945.



# General Reviews

## INTRACRANIAL VASCULAR TUMORS AND MALFORMATIONS

HAROLD H. NORAN, M.D., PH.D.

MINNEAPOLIS

Since the monographic studies of Cushing and Bailey,<sup>1</sup> Lindau,<sup>2</sup> and Bergstrand, Olivecrona and Tonnis,<sup>3</sup> a most extensive literature has accumulated on the subject of vascular tumors of the intracranial cavity. Although much progress has been made in knowledge of the clinical and pathologic features of these lesions, yet much confusion remains, as indicated by the multiplicity of nomenclature and classification. Many different vascular lesions have been recorded under the same title whereas similar lesions have been recorded under a wide variety of titles. Furthermore a large number of the classifications that have been presented for these vascular tumors are conflicting. This almost complete lack of standardization in nomenclature and classification has made the evaluation of the already abundant literature highly difficult.

Nevertheless a comprehensive evaluation of the literature is warranted in order that one may arrive at some correlation between these various nomenclatures and classifications.

### NATURE OF THE VASCULAR TUMORS

There are two main types of vascular tumors. They are referred to as angioma and angioblastoma. Angioma is a tumor composed of blood vessels of adult structure, while angioblastoma is comprised of embryonic vascular channels and proliferating vasoformative cells.

A prodigious amount of discussion has been recorded as to whether or not the tumors called angioma are true neoplasms or mere malformations. Cushing and Bailey,<sup>1</sup> Raney and Courville,<sup>4</sup> Levine,<sup>5</sup> Luschka,<sup>6</sup> and Dandy<sup>7</sup> were among those who expressed the belief that these

tumors represent congenital anomalies. Globus<sup>8</sup> and Laidlow and Murray<sup>9</sup> postulated that angioma arose from a vestigial structure or a phylogenetic remnant, such as the gill arch. Schuck<sup>10</sup> agreed that it was the result of faulty development but felt that it was capable of spontaneous and autonomous expansile growth. A number of authors have employed the term "hamartoma" instead of "angioma." Schmolck,<sup>11</sup> and Albrecht<sup>12</sup> defined the term "hamartoma" as designating a tumor-like anomaly, thereby evading the issue. Turner and Kernohan<sup>13</sup> also used this term but regarded the lesion as a pure malformation. They stressed the belief that angioma increases in size by enlargement of its individual vessels rather than by actual growth. Moolten,<sup>14</sup> on the other hand, expressed the opinion that hamartoma is capable of becoming cancerous and regarded angioblastoma as hamartoma rather than angioma. Bergstrand and his co-authors<sup>3</sup> concluded that angioma is a true tumor since it often becomes a space-consuming lesion. Schwartz<sup>15</sup> agreed since he regarded it as an autonomous new growth of tissue, thereby fulfilling Ewing's definition of a tumor. Virchow,<sup>16</sup> Ribbert,<sup>17</sup>

4 Raney, R. B., and Courville, C. B. *Bull. Los Angeles Neurol. Soc.* **2**: 104, 1937.

5 Levine, V. *Arch. Path.* **15**: 340, 1933.

6 Luschka, H. *Virchows Arch. f. path. Anat.* **6**: 458, 1854.

7 Dandy, W. E. *Arch. Surg.* **17**: 715, 1928.

8 Globus, J. H. *Arch. Neurol. & Psychiat.* **38**: 677, 1937.

9 Laidlow, G. F., and Murray, M. R. *Am. J. Path.* **9**: 827, 1929.

10 Schuck, P. *Ueber das Wesen und die Entstehung der Angioma arteriale racemosum*, Inaug. Dissert., Berlin, O. Francke, 1885.

11 Schmolck, W. *Ueber ein sogenanntes Rankenangioma des Gehirns*, Inaug. Dissert., Wurzburg, F. Staudenraus, 1912.

12 Albrecht, E. *Verhandl. d. deutsch. path. Gesellsch.* **7**: 153, 1904.

13 Turner, O. A., and Kernohan, J. W. *Arch. Neurol. & Psychiat.* **46**: 444, 1941.

14 Moolten, S. E. *Arch. Int. Med.* **69**: 589, 1942.

15 Schwartz, C. W. *Am. J. Roentgenol.* **41**: 881, 1939.

16 Virchow, R. *Die krankhaften Geschwulste*, Berlin, A. Hirschwald, 1863, vol. 3, lect. 25, p. 460.

From the Department of Neuropsychiatry and the Department of Pathology, University of Minnesota Medical School.

1 Cushing, H., and Bailey, P. *Tumors Arising from the Blood Vessels of the Brain. Angiomatous Malformations and Hemangioblastomas*, Springfield, Ill., Charles C. Thomas, Publisher, 1928.

2 Lindau, A. *Acta path. et microbiol. Scandinav.* **3**: 1, 1926.

3 Bergstrand, H., Olivecrona, H., and Tonnis, W. *Gefassmissbildungen und Gefassgeschwulste des Gehirns*, Leipzig, Georg Thieme, 1936.

Simmonds<sup>18</sup> and Heine<sup>19</sup> also expressed the opinion that angioma is a real neoplasm

In general, most writers view the tumors called angioblastoma as true neoplasms exhibiting cellular proliferation. Turner and Kernohan,<sup>13</sup> however, have stated that angioblastoma might arise from hamartoma or a congenital anomaly. Bergstrand and his co-workers<sup>3</sup> cited an example of angioblastoma in a stillborn infant as evidence favoring the belief that the lesion is congenital. They further pointed out the frequent concomitant occurrence of obvious congenital anomalies in other organs.

From this discussion it becomes apparent that at present it is impossible to state definitely whether angioma or even angioblastoma is a simple or a cancerous growth, they present characteristics of both malformations and neoplasms. The term "hamartoma" is not satisfactory, for it has been used to designate a miscellaneous group of lesions and adds nothing to present knowledge of vascular tumors.

#### CLASSIFICATION OF THE VASCULAR TUMORS AND MALFORMATIONS

A review of the classifications in the literature clearly portrays the lack of agreement regarding the basic structure of the individual lesions and also points out the frequency of conflicting nomenclatures. In general, these classifications have lacked uniformity and thus have served merely to add further confusion to an already extensive literature.

The classification offered by Cushing and Bailey has been widely quoted and is generally followed rather closely, especially in this country. It is as follows:

##### I Vascular malformations

- 1 Telangiectasis (a group of capillaries)
- 2 Angioma venosum (lesions composed principally of veins)
- 3 Angioma arteriale (lesions containing vessels resembling arteries as well as veins)

##### II Hemangioblastoma (angioblastoma)

- 1 Cystic type (associated with cyst formation)
- 2 Solid type (not associated with cyst formation)

Each of these two types was again classified into capillary, cellular and cavernous varieties according to the microscopic features.

The presence of brain tissue between the component vessels of angioma was of paramount importance in leading these investigators to conclude that it was an anomaly or a malformation

rather than a neoplasm. Since material for histologic study was not available in most of their cases, the structure of the vessels comprising the angioma was ascertained merely from the resultant clinical manifestations or by a superficial examination of the lesion at operation. They made no distinction between the angioma which was confined to the meninges and the angioma which extended deeply into the cerebral parenchyma, probably because of the paucity of autopsy studies. The lesion comprised of only a few anomalous vessels was not separated from the one composed of an extensive congeries of vessels, replacing much of the brain parenchyma. Moreover, the authors inferred that the well known cavernous type of angioma, which consists of closely packed dilated vascular sinuses, was a variety of angioblastoma, and consequently they provided no place for this type of lesion in their classification.

Turner and Kernohan<sup>13</sup> in studying a large series of vascular neoplasms of the spinal cord suggested the following classification:

##### I Vascular malformations

- 1 Telangiectasis
- 2 Angioma or hamartoma
  - (a) Angioma venosum
  - (b) Angioma arteriovenosum or angioma arteriale

##### II Vascular neoplasms

- 1 Capillary
  - (a) Capillary hemangioma
  - (b) Hemangioendothelioma
  - (c) Capillary hemangioblastoma
- 2 Cavernous
  - (a) Cavernous hemangioma
  - (b) Cavernous hemangioblastoma
- 3 Hemangiosarcoma

Except for the use of the term "hamartoma," the first part of this classification is similar to that of Cushing and Bailey. They attempted to divide the tumors termed angioblastoma into groups manifesting various degrees of histologic malignancy. The capillary hemangioma consisted mainly of capillaries. The hemangioendothelioma was comprised not only of capillaries but of a few proliferating endothelial cells in the intervascular areas. In capillary hemangioblastoma the intervascular regions contained many more proliferating cells. The cavernous hemangioma and hemangioblastoma showed similar structures except that they contained numerous cavernous spaces. The hemangiosarcoma was a vascular tumor showing evidence of rapid growth. The terminologic scheme of the second group differs from that generally accepted, for all these lesions, as a rule, are regarded merely as angioblastoma. The true cavernous angioma was omitted as in

<sup>17</sup> Ribbert, H. Virchows Arch f path Anat **151** 381, 1898

<sup>18</sup> Simmonds, M. Virchows Arch f path Anat **180** 280, 1903

<sup>19</sup> Heine, L. Ztschr f Augenh **51** 1, 1923

the classification of Cushing and Bailey. Finally, no distinction was made between the purely meningeal lesion and that involving the parenchyma.

Rienhoff's<sup>20</sup> classification has not been widely used.

- 1 Arterial aneurysm (tumor consisting of arteries)
- 2 Venous aneurysm (tumor comprised of veins)
- 3 Arteriovenous aneurysm (tumor containing both arteries and veins)

The use of the term "aneurysm" is not satisfactory, for it confuses the tumors ordinarily termed angioma with simple aneurysms of the cerebral arteries as well as with the traumatic fistula between the internal carotid artery and the cavernous sinus. The latter is often termed an arteriovenous aneurysm.

Dandy published the following classification, which is frequently referred to in the literature.

- I Arteriovenous aneurysm
- II Venous abnormality
  - 1 Venous abnormality in the brain or venous anomaly
  - 2 Venous abnormality in the dura
  - 3 Angioma
    - (a) Cyst with angioma in the wall (actually angioblastoma)
    - (b) Cavernous angioma
    - (c) Plexiform angioma (venous angioma)

This classification is not based on the actual pathologic structure of the lesions. Among the venous abnormalities of the brain and the dura were included lesions comprised of anomalous veins showing little increase in their size and number. Under angioma were listed venous tumors (plexiform angioma), but for some reason the arteriovenous tumors were called arteriovenous aneurysms. Dandy incorrectly regarded the cystic form of angioblastoma as angioma and left no place in his classification for the solid or only partly cystic angioblastoma.

Bergstrand, Olivecrona and Tonnis<sup>3</sup> worked out a classification which has appeared in many publications and is often followed in foreign literature.

- 1 Angioma cavernosum
- 2 Angioma racemosum (a grapelike cluster of vessels)
  - (a) Telangiectasis
  - (b) Sturge-Weber syndrome (a condition consisting of an angioma of the surface of the brain with calcification of the underlying cortex and an ipsilateral facial nevus)
  - (c) Angioma racemosum arteriale (a grapelike cluster of vessels exclusively arterial)

- (d) Angioma racemosum venosum (a congeries of vessels resembling veins)
- (e) Arteriovenous aneurysm (a mass of inextricable vessels resembling arteries and veins)

3 Angioreticuloma (angioblastoma)

4 Angioglioma (a combination of glial and vascular elements)

This is the most comprehensive classification found in the literature. It is questionable, however, if telangiectasis should be regarded as racemose angioma. In addition, it seems unwise to place Sturge-Weber disease in this same group, for the calcification and degeneration of the involved cortex are the most significant lesions both clinically and pathologically. These authors questioned the existence of the arterial racemose angioma. As in other classifications, no distinction was made between the lesions limited to the meninges and those involving the brain parenchyma. The term "arteriovenous aneurysm" was also employed here for arteriovenous angioma. The name "angioreticuloma" was certainly no improvement over the more widely accepted "angioblastoma".

Russel<sup>21</sup> offered the following classification.

- 1 Cirroid aneurysm (lesions generally termed racemose angioma)
- 2 Capillary hemangioma (angioblastoma)
- 3 Capillary telangiectasis (telangiectasis)
- 4 Cavernous hemangioma

This classification is more general than those already listed. The nomenclature, however, is confusing. The term "capillary hemangioma" is certainly more suggestive of telangiectasis, for example, than of angioblastoma.

The classification used by Virchow is often mentioned in the literature.

- 1 Cavernous angioma
- 2 Angioma simplex (telangiectasis)
- 3 Telangiectatic tumor formation (false angioma)
- 4 Angioma racemosum

There is a definite resemblance to the better known classification presented by Bergstrand, Olivecrona and Tonnis. Angioblastoma would probably be classified as false angioma.

The following classification of vascular lesions was published by Bort<sup>22</sup>.

- 1 Aneurysm (a simple aneurysm of an artery—not an angioma)
- 2 Serpentine aneurysm (angioma racemosum arteriale or an extensive simple aneurysm)

<sup>21</sup> Russel, D. Proc Roy Soc Med **24** 383, 1931.

<sup>22</sup> Bort. Beitrag zur Kenntnis des Angioma racemosum des Gehirns, Inaug Dissert, Leipzig, Arb a d Geb d path Anat Inst zu Tubingen, 1920.

<sup>20</sup> Rienhoff, W F, Jr. Bull Johns Hopkins Hosp **35** 271, 1924.

- 3 Serpentine angioma (venous or arteriovenous angioma)
- 4 Arterial racemose angioma (a congeries of arteries and probably veins)
- 5 Venous racemose angioma (a plexus of veins)
- 6 Racemose varix (probably similar to the foregoing type, being composed of a congeries of varices but no doubt also includes cavernoma)
- 7 Varix (a simple dilatation of a vein or a venous anomaly)

This classification is extremely difficult to follow, as Bort's description of the various types of lesions is not clear. The use of the terms "serpentine aneurysm" and "serpentine angioma" is confusing. Although the racemose varix is probably comprised largely of cavernous angioma the term is readily confused with "venous racemose angioma." This is the only classification that clearly recognizes the most simple venous anomaly or varix. Bort completely neglected to mention telangiectasis and angioblastoma.

Benda<sup>23</sup> classified the venous tumors as follows

- 1 Phlebectasia (a diffuse widening of a vein)
- 2 Varicosity (a diffuse irregular dilatation with circumscribed ampullar or saclike formations)
- 3 Venous angioma (a circumscribed conglomeration of veins)

This classification deals only with lesions comprised of veins. Phlebectasia and varicosity are the more simple venous anomalies which differ only in the distribution of the vascular dilatations and are similar to the varix in Bort's classification.

*Vascular Tumors of the Meninges*—These lesions are of two general types: one is angiomatous meningioma, which is a meningeal tumor composed of adult vascular elements including arteries, veins, capillaries or cavernous spaces, the second is angioblastic meningioma, which has a structure similar to angioblastoma as found within the cerebellum. Because of the structural similarity of these angioblastic lesions of the meninges to the parenchymal lesions they were classified with the vascular tumors by Bailey and Ford<sup>24</sup>. Cushing and Eisenhardt,<sup>25</sup> on the other hand, regarded them as meningioma basing their opinion on the gross and clinical features rather than on the microscopic characteristics.

Globus<sup>8</sup> classified the angiomatous lesions of the meninges with meningioma, while Cushing

and Bailey<sup>1</sup> as well as others, included them with cerebral angioma probably because their material was operative and no separation was possible in the absence of postmortem studies.

Because of these conflicting opinions regarding the nature of the vascular meningeal lesions, a brief discussion of their classification is indicated. Cushing and Eisenhardt<sup>25</sup> listed the angioblastic meningeal tumors under their meningioma type IV, which was divided into three variants as follows:

- Variant 1 Incompletely differentiated, with mitoses (It is a cellular tumor showing histologic evidence of rapid growth)
- Variant 2 Transitional between the meningothelial type and angioblastoma (It shows transitions toward the meningothelial meningioma)
- Variant 3 Angioblastoma (capillary or cellular) (It has the typical structure of angioblastoma)

All the lesions revealed a predominating angioblastic structure as well as a typical network of reticulum. No mention was made of the angiomatous meningioma.

Courville and Abbott<sup>26</sup> offered the following classification:

- 1 Meningeal angioblastoma
- 2 Transitional angioblastoma
- 3 Angioblastic meningioma
- 4 Combined meningotheiomatous-angioblastic meningioma

This classification is difficult to follow because of its redundancy. Certainly the combined meningotheiomatous-angioblastic type is actually the transitional angioblastoma. Such a separation results only in confusion. Moreover, this classification is similar to that of Cushing and Eisenhardt<sup>25</sup> and therefore serves only to complicate the literature.

Globus<sup>8</sup> classified the vascular meningeal tumors with meningioma and subdivided them into the angioblastic and the angiomatous type. His classification is as follows:

Meningioma piale (pial or vascular meningioma)

- 1 Hemangioendotheliomatous subtype (angioblastic meningioma also includes angioblastoma situated within the neural parenchyma)
- 2 Hemangiomatous subtype (angiomatous meningioma)

This is the most comprehensive classification of the vascular meningeal tumors, for it also includes those comprised of adult vessels. This classification of the intracerebral or intracerebellar angioblastic tumors with meningioma is

23 Benda, C, in Henke, F and Lubarsch O. Handbuch der speziellen pathologischen Anatomie, Berlin, Julius Springer, 1924, vol 2

24 Bailey, O T, and Ford, R. Am J Path 18 1, 1942

25 Cushing, H and Eisenhardt, L. Meningiomas, Springfield, Ill, Charles C Thomas, Publisher, 1938

26 Courville, C B, and Abbott, K H. Bull Los Angeles Neurol Soc 5 47, 1940

not in keeping with the present day concept of cerebral tumors

#### PATHOLOGIC STUDY OF THE INDIVIDUAL VASCULAR LESIONS

From a survey of the literature it becomes obvious that there are a number of vascular lesions occurring within the intracranial cavity whose structural characteristics suggest that they might be distinct entities. The available literature concerning these lesions is most conflicting and offers little help in suggesting some orderly manner of thinking about them. Thus, in order to arrive at a satisfactory classification, one should be comprehensive and consider not only the pathologic structure of these lesions, which shows overlapping of many types but also the clinical features. Although the latter are obviously important space will permit no more than a brief reference to the clinical characteristics of each lesion. Consequently, this paper will be restricted principally to pathologic aspects. It seemed possible that such a broad consideration when applied to the extensive available literature and augmented by a review of the cases observed in the departments of neuropsychiatry and pathology of the University of Minnesota Medical School might result in some success in suggesting a practical method of understanding these vascular lesions from a clinicopathologic point of view. In order to begin with a most unbiased approach, each type of intracranial vascular tumor will be considered separately and individually and then an attempt will be made to find some orderly classification based on the observations. The following lesions are the ones considered in this review

- 1 Telangiectasis (dilated capillaries)
- 2 Rendu-Osler disease (familial telangiectasia or generalized angiomatosis)
- 3 Cerebral varix (simple anomalous veins similar to varicosities)
- 4 Sinus pericranii (vascular tumor of the forehead or the scalp communicating with the superior sagittal sinus)
- 5 Cavernoma or cavernous angioma (closely packed large vascular spaces)
- 6 Venous (racemose) angioma (cluster of vessels resembling veins)
- 7 Arterial (racemose) angioma (a congeries of arteries)
- 8 Arteriovenous (racemose) angioma (a congeries of arteries and veins)
- 9 Angiomatous meningioma (adult blood vessel tumor of the meninges)
- 10 Sturge-Weber disease (characterized by a facial nevus associated with cerebral calcification and angiomatosis of the overlying meninges)

- 11 Angioblastoma (primarily a cerebellar tumor composed of embryonic vascular elements)
- 12 Lindau's (von Hippel's) disease (angioblastoma of the retina and the nervous system)
- 13 Angioblastic meningioma (embryonic vascular tumor of meninges)
- 14 Angiosarcoma (angioblastic tumor showing rapid growth)
- 15 Angioglioma (a combination of adult vascular and glial elements)

The material in the departments of neuropsychiatry and pathology of the University of Minnesota Medical School consists of 39 cases of vascular tumors. A pathologic investigation of the lesions was made in all but 2 cases. In these 2 instances, a case of sinus pericranii and a case of Sturge-Weber disease the diagnosis was obvious without pathologic studies. The following staining techniques were employed in determining the anatomic structure of the tumors: hematoxylin and phloxine stain, Mallory-Heidenhain azocarmine stain, Holzer stain for glia, Nissl stain, Perldian stain for reticulum, Bodian silver stain for axons, Weigert-Van Gieson and Weigert elastic tissue stains.

#### TELANGIECTASIS

*Introduction*—The term "telangiectasis" designates a small lesion composed of a collection of dilated capillaries. A number of cases of telangiectasis have been reported in the literature, and, surprisingly enough, little conflict of terminology is found, most authors using the term "telangiectasis." However, vascular tumors which are distinctly different structurally from this lesion have been described as telangiectasis by Michael and Levin,<sup>27</sup> Oppenheim,<sup>28</sup> Broeckaeft<sup>29</sup> and others. A number with a suggestion of a transitional tendency toward cavernous angioma appeared under the title of cavernoma or cavernous angioma, and a few probably were true transitional forms (Schmidt<sup>30</sup>, Beitzke<sup>31</sup>, Baum<sup>32</sup>, Schweyer<sup>33</sup>, Uyematsu<sup>34</sup>, Schley<sup>35</sup>, Malamud<sup>36</sup>).

27 Michael J C, and Levin, P M. *Arch Neurol & Psychiat* **36** 514, 1936.

28 Oppenheim, H. *Neurol Centralbl* **32** 3, 1913.

29 Broeckaeft, J. *Bull. Acad. roy. de med. de Belgique* **22** 360, 1908.

30 Schmidt, O. *Beitr. z. klin. Chir.* **118** 178, 1920.

31 Beitzke, H. *Charite-Ann.* **31** 360, 1906.

32 Baum. *Munchen med. Wchnschr.* **58** 411, 1911.

33 Schweyer, H. *Arch. f. d. Geb. d. path. Anat. u. Bakt.* **8** 145, 1914.

34 Uyematsu, S. *J. Nerv. & Ment. Dis.* **52** 388, 1920.

35 Schley, W. *Virchows Arch. f. path. Anat.* **265** 665, 1927.

36 Malamud, W. *Ztschr. f. d. ges. Neurol. u. Psychiat.* **97** 651, 1925.

The lesion is almost always silent clinically, but on rare occasions symptoms may be produced as a result of a rupture of one of the small vessels (Michael and Levin<sup>27</sup>). A few published cases suggest hereditary factors, however, the evidence for this is weak (Kufs<sup>37</sup>, Oppenheim<sup>28</sup>, Michael and Levin<sup>27</sup>).

**Gross Pathology**—Telangiectasis is a rather small, poorly circumscribed lesion which may be found in almost any part of the nervous system. It is common in the cerebrum, where it may be situated within the cortex, the white matter or the basal nuclei. It usually occurs deep within the cerebral parenchyma and rarely reaches the brain surface. An exception was reported by Jaffé,<sup>38</sup> in which multiple telangiectasis was encountered in the meninges and the spinal nerve rootlets. Telangiectasis is also found within the brain stem, where it shows a predilection for the pons, according to Ohlmacher,<sup>39</sup>

TABLE 1—Cases of Intracranial Telangiectasis Studied in the Departments of Neuropsychiatry and Pathology of the University of Minnesota Medical School

Case	Sex	Age, yr	Symptoms	Gross Appearance	Location
1	M	51	Silent	Resembled petechiae	Frontal lobe
2	M	60	Silent	Hemorrhagic lesion	Globus pallidus
3	M	57	Silent	Focus of tiny hemorrhages	Temporal lobe
4	M	57	Silent	Conglomeration of small engorged vessels	Basal ganglia and midbrain

Schley,<sup>35</sup> Sommerfelt,<sup>40</sup> Leyser<sup>41</sup> and others. In a few cases it has been reported located within the spinal cord (Claude and Loyez<sup>42</sup>, Hadlick<sup>43</sup>). It usually occurs as a single isolated lesion, but multiple involvement is not unusual. In the latter case the lesions may be restricted to one relatively localized region of the brain or may be widely disseminated. As many as 30 lesions have been discovered in a single patient (Huebschmann<sup>44</sup>),

The lesion is sometimes rather inconspicuous and might be readily overlooked. Its appearance in the cases tabulated here most commonly sug-

gested a focal collection of petechiae. Occasionally one can grossly identify the tiny vascular spaces filled with blood (case 4). Even though the lesion strongly resembles petechiae, rupture of the vessels with extravasation of blood is extremely rare, and only 1 case of this kind has been found recorded in the literature (Michael and Levin<sup>27</sup>).

**Microscopic Anatomy**—The lesion consists of a collection of numerous engorged capillaries or cavernous spaces which are always separated by various amounts of relatively normal brain tissue. The capillaries may be small, but larger cavernous spaces may be prominent. Regardless of the size of the vessel, its wall consists of a single layer of flat endothelium, which occasionally is surrounded by a thin rim of collagenous tissue. Smooth muscle and elastic elements are never present. The vessels, as a rule, are not so numerous that they replace any significant amount of brain tissue or produce any notable alterations in the regional neural parenchyma.

RENDU-OSLER DISEASE

**Introduction**—Rendu-Osler disease is a rare familial condition in which telangiectatic lesions are disseminated over the skin and may involve various internal organs. The nervous system generally is not implicated, but a few cases of neural involvement have been reported. Therefore, it becomes necessary to review this syndrome briefly.

A number of terms have been used for this disease. It has most often been referred to as familial telangiectasia or generalized angiomatosis. Although it was first described by Sutton<sup>45</sup> in 1864, it was named after Rendu<sup>46</sup> and Osler,<sup>47</sup> who published their cases at a later date.

**Pathologic Features**—The individual lesions are quite typical of telangiectasis regardless of their location. The lesions of the skin are commonly present at birth and increase with age. They are red to purple and vary considerably in size but seldom do they exceed a few millimeters in diameter. Telangiectasis commonly involves the mucous membranes of the respiratory and gastrointestinal tracts and the urogenital system. Hemorrhage from the lesions is common, and the clinical symptoms are the result of this bleeding.

37 Kufs, H. Ztschr f d ges Neurol u Psychiat 113 651, 1928  
38 Jaffe, R. H. Arch Path 7 44, 1929  
39 Ohlmacher, A. P. J Nerv & Ment Dis 26 395, 1899  
40 Cited by Bergstrand, Olvecrona and Tonnis.<sup>3</sup>  
41 Leyser, E. Monatschr f Psychiat u Neurol 51 83, 1922  
42 Claude, H., and Loyez, M. Rev neurol 19 181, 1911  
43 Hadlick, R. Virchows Arch f path Anat 172 429, 1903  
44 Huebschmann. Deutsche Ztschr f Nervenhe 72 205, 1921

45 Sutton, H. G. M. Mirror, London 1 769, 1864  
46 Rendu. Bull et mem Soc med d hôp de Paris 13 731, 1896  
47 Osler, W. Bull Johns Hopkins Hosp 12 333, 1901

Lesions of the central nervous system are silent clinically unless they produce intracerebral or subarachnoid bleeding. Cases of the disease with verified cerebral involvement have been recorded by Archer<sup>48</sup> and Goldstein<sup>49</sup>. Generally there are many persons in the family for several generations afflicted with this condition.

#### CEREBRAL VARIX

*Introduction*—This lesion consists of abnormal, dilated, thin-walled vessels situated either within the brain substance or on its surface. Schaltenbrand<sup>50</sup> reviewed the literature and was able to find reports of only 13 cases in addition to his own. The vascular abnormalities are most frequently referred to as varices or varicosities. Dandy<sup>7</sup> in reporting cases of varices of the

TABLE 2—Cases of Cerebral Varices Studied in the Departments of Neuropsychiatry and Pathology of the University of Minnesota

Case	Sex	Age, Yr	Symptoms and Findings	Gross Appearance	Location
5	F	53	Silent	Not visible	Subcortical white matter of cerebrum
6	F	47	Headache, confusion, paresis of left upper limb, stupor	Subarachnoid bleeding, a large collection of varices with vascular channels connecting with the straight sinus	Surface of right occipital lobe
7	F	75	Vomiting, diplopia, visual hallucinations, papillitis, partial deafness, disorientation, stupor	Ventricular and subarachnoid hemorrhage, varices overlooked on gross examination	Cerebellum and region of pineal gland

superior sagittal sinus incorrectly called the varices dural aneurysms, while Cushing and Bailey<sup>1</sup> classified their 2 cases with cases of venous angioma. Benda<sup>23</sup> advanced the title "phlebectasia."

The lesions may be clinically silent and hence may be discovered accidentally at autopsy (case 5). In many cases, however, clinical symptoms do occur but are usually the result of intracranial hemorrhage or, less commonly, of vascular thrombosis eventuating from the varix.

*Gross Pathology*—Varices are often not visible on gross examination or may frequently be overlooked because of their small size (cases 5 and 7).

<sup>48</sup> Archer, cited by Goldstein, H. S. *Arch Int Med* **27** 102, 1921.

<sup>49</sup> Goldstein, H. S. *Internat Clin* **3** 148, 1930.

<sup>50</sup> Schaltenbrand, W. *Frankfurt Ztschr f Path* **52** 363, 1938.

In a number of cases, however, they are sufficiently large to be seen readily (case 6) and may even measure several centimeters in diameter (Beger<sup>51</sup>). In all the cases tabulated in this section multiple anomalous vessels comprised the lesions. The number of vessels in a lesion is, as a rule, not large, occasionally one encounters a varix consisting of a single large vessel (Guldenarm and Winkler<sup>52</sup>, Pfannenstiehl<sup>53</sup>, Beger<sup>51</sup>, Wohak<sup>54</sup>, Marx<sup>55</sup>). There is no site of predilection. In fact, the varix may occur in any location within the brain or on its surface, frequently involving even the dural sinuses (Wohak<sup>54</sup>, Marx<sup>55</sup>, Dandy<sup>7</sup>). In the latter case there is a large vascular dilatation which may cause partial erosion of the overlying skull and occasionally softening of the adjacent cerebral tissue. The dural sinuses may be involved directly by the varix or may be connected with it by means of anomalous vascular channels (case 6).

The most common complication of the intracerebral varix is an intracranial hemorrhage (cases 6 and 7). Depending on the location of the abnormal vessel which ruptures to produce the bleeding, blood may extravasate into the brain tissue, the subarachnoid space or the ventricular system.

*Microscopic Anatomy*—The cerebral varix shows a rather characteristic histologic picture. It consists of a relatively large thin-walled vessel, usually lined by a single layer of endothelium and encircled by a relatively thin lamina of fibrous connective tissue (fig 1). The latter often undergoes hyaline degeneration. Muscle fibers and elastic elements are generally absent (Schaltenbrand<sup>50</sup>, Esser<sup>56</sup>). These vessels frequently resemble the large vascular spaces of cavernous angioma, but in contrast to the latter the varicose vessels are separated from one another by cerebral parenchyma. This regional cerebral tissue may be intact or may show rather prominent degenerative and reactive alterations.

When muscle or elastic tissue is found in the wall of the varix, these elements are less prominent than in normal veins. A lesion showing this kind of vascular structure presents a vague

<sup>51</sup> Beger, H. *Virchows Arch f path Anat* **231** 439, 1921.

<sup>52</sup> Guldenarm, J. A., and Winkler, C. *Nederl tijdschr v geneesk* **27** 217, 1891.

<sup>53</sup> Pfannenstiehl. *Centralbl f Gynak* **11** 601, 1887.

<sup>54</sup> Wohak, H. *Virchows Arch f path Anat* **242** 58, 1923.

<sup>55</sup> Marx, A. M. *Med Klin* **21** 1612, 1925.

<sup>56</sup> Esser, A. *Verhandl d deutsch path Gesellsch* **20** 411, 1925.



resemblance to racemose angioma. However, in the varices, vessels of relatively typical arterial or venous structure are never seen, and the vessels are less numerous and more uniform in structure than the numerous irregular, most variable elements of racemose angioma. The usual complication in this lesion is a rupture of the varix wall with a resulting cerebral



Fig 1.—Small intracerebral varices. Note the large dilated vascular spaces separated by intact cerebral tissue. The vessel walls are thin, consisting of a flat endothelial lining and a thin layer of fibrous connective tissue. Weigert–Van Gieson stain.

Fig 2.—The characteristic appearance of venous angioma is observed in the cone-shaped mass of angiomatous vessels extending deep into the cerebral hemisphere. Note the large tortuous vessels over the surface of the lesion, which course along the meninges of the neighboring regions of the brain for some distance.

Fig 3.—Cerebellar angioblastoma demonstrating numerous small vascular spaces separated by proliferating endothelial cells. Hematoxylin and eosin stain.

hemorrhage Thrombosis with hemorrhagic infarction was encountered in Amsler's<sup>57</sup> third case and my case 6

The etiologic nature of the intracranial varices has occasioned much speculation Most authors (Anders,<sup>58</sup> Kallenberger,<sup>59</sup> Kaufman,<sup>60</sup> Orth<sup>61</sup> and others) have thought that they resulted from a congenital weakness of the vessel walls, but others have postulated that they eventuated from an earlier venous inflammation (Beger<sup>51</sup>), from prolonged trauma over bony ridges (Amsler<sup>57</sup>, Fisher<sup>62</sup>) or from mechanical factors eventuating from abnormal presentation of the head during birth

### SINUS PERICRANII

*Introduction*—Sinus pericranii is a vascular tumor which, as a rule, protrudes from the forehead and communicates with the superior sagittal sinus Although this lesion is largely outside the skull, it should be considered with the intracranial vascular lesions because of its connection with the superior sagittal sinus and its occasional production of cerebral symptoms For some reason, most authors when discussing the vascular neoplasms of the brain fail to mention this condition

The lesion was first described in 1760 by Percival Pott<sup>63</sup> and later was termed sinus pericranii by Stromeyer<sup>64</sup> Hahn<sup>65</sup> in 1928 reviewed the literature, discovering 84 recorded examples

Clinically, sinus pericranii in many cases produces no symptoms other than the tumor of the forehead The tumor appears early in life and may enlarge slowly over a period of many years It is soft and compressible Anything that increases the intrathoracic or the venous pressure, such as coughing or straining and lowering the head, causes the tumor to enlarge In rare cases, variable cerebral symptoms, including increased intracranial pressure and a cerebral bruit, have been described

*Gross Pathology*—The tabulated case was not studied pathologically, and unfortunately few

careful pathologic studies have been published In a majority of cases the tumor was situated in the midportion of the forehead as in the case tabulated, but almost any point along the superior sagittal sinus as far back as the occipital region has been involved in occasional instances (Wakely) A defect occurs in the underlying cranial bones allowing the lesion to communicate with the adjacent dural sinus The appearance of the actual lesion is that of a congeries of small and large vascular spaces, often resembling cavernous angioma In fact, it is likely that a number were similar to cavernous angioma (Mueller<sup>66</sup>)

*Microscopic Anatomy*—Generally there are numerous thin-walled vascular spaces, which are of irregular contour and are embedded in fibrous connective tissue The spaces are connected with one another by a multiplicity of small anastomotic vascular channels having the structure of capillaries In Wakely's case the intervacular areas were highly cellular, creating an appearance which the author felt was con-

TABLE 3—A Case of Sinus Pericranii\*

Case	Sex	Age	Symptoms and Findings
8	F	4 yr	Enlarging tumor of midline of forehead containing venous blood It was obliterated by injections of a sclerosing solution

\* A report of this case was published previously (Peyton, W Minnesota Med 21:590, 1938)

sistent with angioblastoma Although further pathologic studies must be made before definite conclusions can be drawn regarding the anatomic structure of the tumor, it appears as if it may consist of capillaries, cavernous spaces, arteries or veins and possibly even proliferating angioblasts Furthermore, the clinical symptoms in certain cases suggest that there is an associated intracranial vascular lesion in addition to the communication with the sagittal sinus

### CAVERNOUS ANGIOMA

*Introduction*—Cavernous angioma is a vascular lesion comprised of closely packed dilated vascular spaces which are not separated by brain tissue It is often referred to as cavernoma Dandy<sup>7</sup> in reviewing the literature discovered 44 cases of what he considered to be cavernoma Other authors, however, have felt that Dandy<sup>7</sup> included a number of vascular tumors which were not cavernous angioma but were actually other forms, such as racemose angioma and telangiectasis (Bergstrand and co-workers,<sup>3</sup> among others) Many authors have recorded

<sup>57</sup> Amsler, C Frankfurt Ztschr f Path 11 254, 1912

<sup>58</sup> Anders Beitr z path Anat u z allg Path 64 540, 1918

<sup>59</sup> Kallenberger, W Virchows Arch f path Anat 180 130, 1905

<sup>60</sup> Kaufman Zentralbl f Gynak 37 209, 1897

<sup>61</sup> Orth, J Lehrbuch der speciellen pathologischen Anatomie, Berlin, A Hirschwald, 1887

<sup>62</sup> Fisher, B Beitr z path Anat u z allg Path 27 484, 1900

<sup>63</sup> Pott, P, cited by Hahn<sup>65</sup>

<sup>64</sup> Stromeyer Deutsche Klin 11 160, 1850

<sup>65</sup> Hahn, E Arch Surg 16 31, 1928

<sup>66</sup> Mueller, A Klin Wchnschr 49 1372, 1912

cases which have been generally acceptable—for example, Lorenz,<sup>67</sup> Hadlick,<sup>43</sup> Baum,<sup>32</sup> Stief,<sup>68</sup> Dandy,<sup>7</sup> Schmidt,<sup>30</sup> Rossolimo<sup>69</sup> and Bergstrand and his collaborators,<sup>3</sup> as well as others

The literature on cavernous angioma contains a confusing number of terms, primarily because many of the authors have not understood the basic structure of tumors of this type. Yates and Paine<sup>70</sup> regarded what appeared to be a cavernous lesion as an arteriovenous angioma because they were able to inject it after death through its arterial supply. Cushing and Bailey<sup>1</sup> confused cavernoma with angioblastoma, regarding some examples of the latter as representing a cavernous type of angioblastoma. In none of their cases, however, was a true cavernous tumor described. Lesions of this type have also been placed under the heading of telangiectasis (Michael and Levin<sup>27</sup>)

TABLE 4—Cases of Cavernous Angioma Studied in the Departments of Neuropsychiatry and Pathology of the University of Minnesota

Case	Sex	Age, yr	Symptoms and Findings	Gross Appearance	Location
9	F	36	Jacksonian epilepsy, hemiplegia, cranial bruit, intellectual deterioration	Intracerebral spongy tumors comprised of closely packed dilated and thin walled vessels	One large and 3 small tumors within cerebrum, 1 within cerebellum
10	M	62	Jacksonian epilepsy, hemiparesis, aphasia, increased intracranial pressure	Wedge shaped tumor made up of large dilated blood vessels within brain	Left frontoparietal region
11	M	10	Increased intracranial pressure, somnolence, obesity, polyuria, failing memory	Spongy vascular tumor within ventricular system producing internal hydrocephalus	Third ventricle and hypothalamus

Cavernous angioma produces no characteristic symptoms, in many instances it is entirely silent, while in others it is manifest in a variable picture of recurrent convulsive or focal phenomena. A cranial bruit is a rare finding, but intracranial hypertension is not uncommon.

**Gross Pathology**—In the literature, cavernous angioma is generally described as being situated deep within the brain substance but in all the cases tabulated here it reached the brain surface, and in 2 cases the overlying surface was covered with enlarged tortuous vessels. In the already published cases the tumor was located in order of

frequency within the frontoparietal region, the pons, the vicinity of the third ventricle and the occipital lobes. In the cases tabulated here the tumor was found in the cerebral hemispheres, the third ventricle and the cerebellum, respectively. Cavernous angioma is usually single (cases 10 and 11), but occasionally it may be multiple and widely disseminated (case 9). Other instances of multiple lesions were reported by Riddoch,<sup>71</sup> Federoff and Bogorad<sup>72</sup> and Kufs.<sup>37</sup>

The tumor itself is reddish-brown or bluish and consists of large thin-walled vascular spaces which are closely packed. It is, as a rule, compressible and spongelike, especially when the cavernous vessels are large. The individual vascular spaces vary considerably in size and may be so congested as to obscure the cavernous vessel walls, thereby presenting a superficial resemblance to an intracerebral hemorrhage. The lesion may be quite sharply circumscribed or may merge gradually with the neighboring parenchyma, the vessels in these regions being separated by nerve tissue, in contrast to the observation within the body of the cavernoma.

The lesion replaces the involved area of the brain and often produces atrophy of the regional tissues. This atrophy may in turn create secondary dilatation of the adjacent ventricle. Occasionally cavernoma protrudes into a ventricle, where it obstructs the outflow of cerebrospinal fluid, thereby producing obstructive internal hydrocephalus (case 11). Although rare, there are authentic cases in which vessels in a cavernoma have ruptured with resultant cerebral hemorrhage (Bergstrand and his associates<sup>3</sup>).

**Microscopic Anatomy**—The histologic appearance of cavernous angioma is constant. The tumor is composed entirely of dilated vascular spaces filled with blood. Since there is no cerebral parenchyma separating the vessels, their walls are in direct contact with one another. The thin walls are comprised solely of fibrous connective tissue which is generally largely hyaline. The cavernous lumens are lined with a single layer of flattened endothelium which never shows any significant proliferative changes. Elastic and smooth muscle fibers are almost never observed (cases 9 and 11). Case 10, however, was an exception, for even in the most classic cavernous regions an occasional vessel wall revealed a very thin and delicate elastic membrane adjacent to the intima, as well as a few smooth muscle fibers. Vascular calcifica-

67 Lorenz, F. O. *Cavernoses Angiome des Rückenmarkes*, Inaug. Dissert., Jena, B. Engau, 1901.

68 Stief, A. *Ztschr. f. d. ges. Neurol. u. Psychiat.* **93**: 181, 1924.

69 Rossolimo, G. *Neurol. Centralbl.* **15**: 714, 1896.

70 Yates, A., and Paine, C. G. *Brain* **53**: 38, 1930.

71 Riddoch, G. *Proc. Roy. Soc. Med.* **24**: 382, 1931.

72 Federoff, H., and Bogorad, F. *Ztschr. f. d. ges. Neurol. u. Psychiat.* **94**: 497, 1924-1925.

tion is common (cases 9 and 11), and thrombosis of the cavernous spaces occurs (case 10)

Around the periphery of the lesion certain vessels which are separated by brain tissue may have arterial or venous structure. These probably represent the afferent and efferent vessels of the tumor. The nearby brain tissue often undergoes regressive alterations and even actual softening with concomitant reactive gliosis (case 11)

#### VENOUS ANGIOMA

*Introduction*—Venous angioma is a tumor of the brain comprised of a congeries of vessels structurally resembling veins. Under this title only those venous tumors extending deep into the brain parenchyma are considered, those limited to the meninges covering the cerebral tissues are correctly discussed later as angiomatous meningioma. Such a distinction was usually not made in the literature.

Largely because of the conflicting viewpoints regarding the structure of venous angioma, a rather redundant and confusing nomenclature has appeared in the literature. A few examples of these titles are the following "venous aneurysm," a name which was obviously unsatisfactory (Vincent and Heuyer<sup>73</sup>, Riehoff<sup>20</sup>), "venous anomaly" or "plexiform angioma" (Dandy<sup>7</sup>), and "venous hamartoma" (Turner and Kernohan<sup>13</sup>). Very often tumors of this type are referred to as cirroid angioma or rankenangioma. Completely inaccurate terms are frequently encountered, as in Christiansen's<sup>74</sup> case, in which a venous tumor was entitled cavernous angioma. The term "venous angioma" is the most descriptive name, however, the title "venous racemose angioma" also should be accepted because of its wide use.

Reports of cases have been surprisingly infrequent and often incomplete. The apparent rarity of this venous lesion may be partly explained by the atypical nature of the clinical picture, which rarely permits antemortem diagnosis, and the prolonged clinical course, which makes it most difficult to follow the case to its termination, when an autopsy could be performed. Examples of venous angioma have been reported by Cushing and Bailey,<sup>1</sup> Bergstrand and his associates,<sup>3</sup> Durck,<sup>75</sup> Therman,<sup>76</sup> Orbi-

son,<sup>77</sup> Dowling<sup>78</sup> and Wolf and Brock,<sup>79</sup> as well as many other authors.

The most frequent clinical manifestations are recurring subarachnoid hemorrhage and repeated apoplectic attacks. Much of the confusion regarding the symptoms results from the frequency with which cases of Sturge-Weber disease have been regarded as instances of venous angioma.

*Gross Pathology*—The most classic variety of venous angioma is a wedge-shaped or cone-shaped lesion in which the base of the cone is in contact with the leptomeninges and the tip reaches the ventricle (fig 2). An aggregation of intertwining vessels is usually observed on the surface overlying the involved area. When

TABLE 5—Cases of Venous Angioma Studied in the Departments of Neuropsychiatry and Pathology of the University of Minnesota

Case, Sex, Age	Symptoms and Findings	Gross Appearance	Location
12 M 37 yr	Increased intra cranial pressure, meningeal irritation, aphasia, hemiplegia	Wedge shaped vascular tumor within brain associated with sub arachnoid and intracerebral bleeding and encephalomalacia	Rolandic region of cerebrum
13 M 31 yr	Recurring episodes of meningeal irritation and stupor	Vascular tumor obstructing ventricular system and extending over cerebral base	Third ventricle
14 F 19 days	Spina bifida, paralysis of lower limbs, Arnold Chiari deformity of cerebellum (removed surgically)	Surgical defect and internal hydrocephalus	Cerebellum
15 F 29 yr	Headache, diplopia, writhing movements, extensor rigidity of limbs, ultimate coma	Large tortuous vessels within an intracerebral hemorrhage rupturing into ventricle, associated cerebral aneurysm	Thalamus
16 M 35 yr	Patient found dead	Vascular tumor within brain	Right frontal lobe

the tumor is small, it may extend only to the deeper sulci, in which case the surface of the brain may appear normal or may reveal only a few enlarged vessels resembling varices (case 12). Within the brain there is a tangled mass of large vessels, which are separated from each other by thin layers of cerebral tissue. The resultant appearance somewhat resembles a varicocele (Bergstrand and his collaborators<sup>3</sup>). In rare instances the vessels are less closely arranged, being more sparsely distributed over the involved area (case 15). Occasionally the superficial vessels may communicate freely with

<sup>73</sup> Vincent, C. L., and Heuyer, G. *Rev. neurol.* 1: 509, 1929.

<sup>74</sup> Christiansen, V. *Les tumeurs du cerveau*, Paris, Masson & Cie, 1921.

<sup>75</sup> Durck, H. *Munchen med. Wchnschr.* 54: 1154, 1906.

<sup>76</sup> Therman, E. *Arb. a. d. path. Inst. d. Univ. Helsingfors* 3: 67, 1910.

<sup>77</sup> Orbison, T. J. *J. A. M. A.* 64: 1575, 1915.

<sup>78</sup> Dowling, E. *Bol. y trab., Soc. de cir. de Buenos Aires* 10: 589, 1926.

<sup>79</sup> Wolf, A., and Brock, S. *Bull. Neurol. Inst. New York* 4: 144, 1935.

the dural sinuses (Hyland and Douglas<sup>80</sup>) Cushing and Bailey<sup>1</sup> insisted that the afferent artery of venous angioma is never enlarged, but case 13 in my table 5 shows that this is not always true. The lesion may actually protrude into a ventricle and if large enough may actually fill the ventricle, producing obstructive hydrocephalus (case 13).

Venous angioma is not an expansile tumor, rather it replaces the tissue of the involved area and not uncommonly causes definite atrophy of the region which may be evidenced by considerable dilatation of the nearby ventricle (case 12) and a pool of fluid overlying the shrunken cortex (Dandy<sup>7</sup>). Not infrequently some bleeding results from the tumor, which is most commonly evidenced by a thin layer of subarachnoid blood. Larger hemorrhages also occur either intracerebrally or into the ventricles (cases 12 and 15). Areas of encephalomalacia may be found adjacent to the tumor.

*Microscopic Anatomy*—Most of the vessels in venous angioma structurally resemble normal veins. In rare instances the tumor may consist of venules (case 14) but never of arteries or arterioles, although a few arterioles may be present in scattered areas throughout the tumor. The vessels are usually irregular and bizarre in shape. Their walls are relatively thin but may show considerable variation. Occasionally, however, scattered vessels may present thick walls, while others may reveal marked irregularity within the circumference of a single structure.

The structure of a single vessel is usually constant. The innermost layer consists of a single flat endothelial lining, often associated with a delicate elastic membrane. This endothelium may undergo localized proliferation, producing finger-like projections into the lumen of the vessel. The elastic lamina, although forming fine reduplications, is usually not prominent and is often incomplete or even absent. The principal mural element of the vessels of these tumors consists of fibrous connective tissue, which underlies the endothelial and elastic laminae. These elements are generally of moderate thickness and may be intermixed with a few smooth muscle fibers. However, a prominent muscular layer does not occur in vessels regarded as venous in nature.

The cerebral parenchyma separating the vessels and surrounding the lesion manifests rather marked changes such as demyelination, degenerative alterations of nerve cells, gliosis, encephalomalacia and occasionally hemorrhage.

A number of investigators in reporting on the morphologic anatomy of tumors of this type regarded many of the thin-walled vessels, even those without a well formed lamina muscularis, as arterial in structure merely because a thin elastic membrane was observed. Bergstrand and his collaborators<sup>3</sup> maintained that the vessels were so abnormal that it was impossible to ascertain whether or not they were arteries or veins. That the vessels are abnormal is obviously true; however, in the cases tabulated here it was not difficult to determine the more specific nature of the vessels. Therefore, only the lesion which is composed of vessels principally of venous structure should be regarded as venous angioma.

#### ARTERIAL ANGIOMA (RACEMOSUM)

The name "arterial (racemose) angioma" refers to a vascular tumor consisting exclusively of a conglomeration of coiled arteries. Simmonds<sup>18</sup> favored the name "angioma arteriale serpentinum." Since the title "arterial angioma" has often been employed for lesions containing veins as well as arteries, most of the cases published in the literature actually were cases of arteriovenous angioma. In order to establish the diagnosis of angioma arteriale, it is necessary to prove that the afferent and efferent vessels as well as those comprising the tumor all have arterial structure. If such a criterion is followed, it is questionable whether such a lesion can be found (Bergstrand and his associates<sup>3</sup>), although its occurrence constitutes a theoretic possibility.

#### ARTERIOVENOUS ANGIOMA

*Introduction*—Arteriovenous angioma is a vascular tumor comprised of a tangled plexus of vessels resembling both arteries and veins. A multiplicity of often ambiguous and confusing terms have been employed in the recording of cases. Lesions of this type have been referred to as arterial angioma (Cushing and Bailey<sup>1</sup> and others). Such a name, however, implies that they are comprised entirely of arteries. They also have been designated as arteriovenous aneurysm (Bergstrand and his co-workers,<sup>3</sup> Dandy<sup>7</sup> and others), however, this title is more correctly used in describing the traumatic fistula between the internal carotid artery and the cavernous sinus, which is not considered in this review. Other terms have also been suggested such as "aneurysmal angioma" (Craig<sup>81</sup>), "arteriovenous hamartoma" (Turner and Keino-han<sup>13</sup>), "cirroid aneurysm" (Uiberall<sup>82</sup>) and

81 Craig, W. M. *Am Heart J* 17 40, 1939.

82 Uiberall, H. *Ztschr f d ges Neurol u Psychiat* 124 863, 1930.

80 Hyland, H. H., and Douglas, R. P. *Arch Neurol & Psychiat* 40 1220, 1938.

many others. A few extremely confusing terms have been encountered such as "angioma anastomoticum," "varix arteriale," "varix aneurysmaticus" and "rankenaneurysm." Although the term "arteriovenous angioma" rarely appears in the recorded publications, it seems the most consistent with the pathologic structure and therefore should receive preference over other titles. It should also be mentioned that even though a rather large number of cases have been recorded, in very few of the descriptions has the anatomic structure been stressed.

The most characteristic clinical picture consists of convulsive seizures associated with focal

TABLE 6—Cases of Arteriovenous Angioma Studied in the Departments of Neuropsychiatry and Pathology of the University of Minnesota

Case, Sex, Age	Symptoms and Findings	Gross Appearance	Location
17 M 26 yr	Deformed head, obesity, hypoplastic genitalia, strongly pulsating carotid arteries, optic atrophy, exophthalmos, positive Babinski signs, cerebellar symptoms, right hemihypesthesia	Very large tortuous vessels coursing diffusely over surface of brain and along ventricles, large expansile vascular tumor producing obstructive internal hydrocephalus	Cerebellum
18 F 56 yr	Petit and grand mal convulsions, attacks of aphasia, right hemiparesis and hemianesthesia	Extensive wedge shaped vascular tumor within brain substance compressing adjacent ventricle, part of tumor filled with hemorrhage	Greater part of left cerebral hemisphere above lateral ventricle
19 F 46 yr	Grand mal convulsions, temporal fits, emotional and neurotic disturbances and intellectual deterioration	Subarachnoid bleeding, large angiomatous vessels over cerebral surface, large intracerebral hemorrhage containing numerous large angiomatous vessels	Left temporal lobe
20 M 57 yr	Right sided Jacksonian epilepsy, hemiparesis, hemihypesthesia, aphasia	Large vascular tumor within brain containing diffuse hemorrhage which had extravasated into subarachnoid space and ventricles	Left parietal and occipital regions

symptoms recurring for many years. Contrary to the generally accepted conception, a cranial bruit and increased intracranial pressure are not common findings. Moreover, roentgenographically demonstrable calcification is more common within lesions of this type than in purely venous lesions.

**Gross Pathology**—Arteriovenous angioma occurs most commonly in the cerebral hemispheres and only rarely in the cerebellum (case 17). In appearance the lesion is so similar macroscopically to venous angioma that a differential diagnosis cannot be made by gross examination alone. Like the venous, the arteriovenous tumor often occurs as a wedge-shaped lesion extending deep into the brain parenchyma. Although it fre-

quently only replaces the involved tissue, resulting in atrophy of the region, in certain instances it behaves as an expansile growth compressing the surrounding structures (case 17). This occasional expansile nature is not encountered in venous angioma.

As a rule, the tumor is confined to one region of the brain (case 19) but may in certain instances extend throughout a cerebral hemisphere (case 18). Usually the entire surface of the involved region is covered with a dense plexus of angiomatous vessels. Not infrequently large abnormal vessels are observed over extensive areas of the brain surface not directly related to the principal vascular tumor. These vessels as well as those overlying the angioma itself often connect with enlarged tortuous vessels situated over the basilar structures. The latter vessels, in rare cases, may course along the optic nerve to reach the orbit and even the retina, according to Heitmüller,<sup>83</sup> Kreutz,<sup>84</sup> Rotgans and Winkler<sup>85</sup> and others. Exophthalmos may result, as in case 17. Occasionally in cases of arteriovenous angioma, large tortuous angiomatous vessels are observed involving the scalp. A moderate degree of dilatation and hypertrophy of the artery supplying the region involved by the angioma occurs more frequently in the arteriovenous than in the venous tumor.

Some portion of the angioma is usually in contact with one of the ventricles, occasionally producing considerable compression and even obstruction of the involved portion of the ventricle. Obstructive hydrocephalus may result in this way, but more frequently ventricular dilatation eventuates from the diffuse parenchymal atrophy. At times large angiomatous vessels may be observed coursing along the ventricular walls at a great distance from the main lesion (case 17).

In many cases some form of intracerebral hemorrhage eventuates from the rupture of one of the angiomatous vessels (cases 18, 19 and 20). Bleeding may be so extensive as to obscure much of the vascular nature of the tumor, creating the appearance of a cerebral hemorrhage (cases 18 and 19). Subarachnoid and intraventricular bleeding are both common. Occasionally the rupture of a cerebral hemorrhage into a ventricle produces a cyst communicating with the ventricle (Sorgo<sup>86</sup>). Grossly visible vascular calci-

<sup>83</sup> Heitmüller, G. H. J. A. M. A. **42**: 648, 1904.

<sup>84</sup> Kreutz, A. Wien med Wchnschr. **53**: 1726, 1903.

<sup>85</sup> Rotgans, J., and Winkler, C. Aneurysme racineux de la pie-mere de l'hémisphère gauche. Exophthalmie pulsatile à droite. Trepanation. Hémiplegie transitoire. L'état actuel de la chirurgie nerveuse, Paris, Chippault, 1902, p. 695.

<sup>86</sup> Sorgo, W. Zentralbl. f. Neurochir. **3**: 64, 1938.

fication is encountered with much greater frequency than in cases of venous angioma

*Microscopic Anatomy*—This variety of vascular tumor is comprised of a collection of adult blood vessels which are separated from one another by brain tissue. The general histologic picture is similar to that described previously for venous angioma. Scattered throughout arteriovenous angioma, however, there are numerous vessels clearly resembling arteries. In 3 of the 4 cases tabulated here (17, 18 and 20), more than half of the vessels were arterial, while in only 1 case (19) were veins more numerous, yet even in this case vessels resembling arteries were prominent throughout the tumor.

As the vessels of arterial structure are the only morphologic feature by which arteriovenous angioma can be distinguished from venous angioma, the microscopic anatomy of only these vessels requires description. They tend to be round or oval but may be irregular in contour. Their walls are generally thick, with the individual vessels revealing little variation in their mural thickness.

The most characteristic feature of such a vessel is the presence of a relatively thick layer of smooth muscle comprising much of the wall. No vessel should be considered arterial without this type of tunica media. The lumen of the vessel is lined by a single layer of endothelium which may produce partial occlusion of the lumen through its proliferation. A prominent thick and wavy internal elastic membrane is usually present around the entire circumference of the vessel. It frequently reveals multiple reduplications. Such an elastic intima may be absent in an occasional arterial vessel and hence is of less value than the muscular lamina in determining the vessel's arterial structure.

Degenerative changes in the vessel wall are not prominent, but calcification of scattered vessels is the rule. The parenchyma surrounding the lesions and separating the individual vessels shows considerable degenerative alterations, such as disease of nerve cells, demyelination, encephalomalacia and gliosis. Extensive hemorrhages and large cerebral softenings are not uncommon.

ANGIOMATOUS MENINGIOMA

*Introduction*—Angiomatous meningioma is a vascular tumor of the meninges comprised of various combinations of adult blood vessels, such as arteries, veins, cavernous spaces or capillaries. The lack of involvement of the underlying cerebral tissue differentiates this type of vascular tumor from the deeper, parenchymal angioma.

The literature is most meager as regards angioma of the meninges, only a few verified cases

having been reported, such as those recorded by Worster-Drought and Ballance<sup>87</sup> and Globus.<sup>8</sup> Globus<sup>8</sup> was the only author to use the term "angiomatous meningioma." Cushing and Bailey<sup>1</sup> expressed the opinion that in most of their cases of venous angioma the lesion, which they called a superficial serpentine lesion, was restricted to the cerebral meninges, however, they were unable to prove this since none of their cases was studied pathologically. It was probably for this reason that angiomatous meningioma was not included in their classification.

Angiomatous meningioma may occur in Sturge-Weber disease as a concomitant lesion, however, in this condition it is not always present and

TABLE 7—Cases of Angiomatous Meningioma Studied in the Departments of Neuropsychiatry and Pathology of the University of Minnesota

Case No., Sex, Age	Symptoms and Findings	Gross Appearance	Location
21 F 65 yr	Delirium, coma, vomiting, respiratory difficulty	Thick layer of subarachnoid hemorrhage	Surface of cerebellum
22 M 59 yr	Symptoms of increased intracranial pressure, left internal squint, right ptosis and facial palsy, ataxia, pyramidal tract signs, dysphagia, respiratory difficulty	Extensive subarachnoid bleeding, filled with a network of large tortuous vessels	Surface of cerebellum and right side of pons
23 M 51 yr	Increased intracranial pressure, suboccipital headache, paresthesias of upper limbs, left cerebellar findings, signs of involvement of right pyramidal tract	Layer of extravasated blood containing large angiomatous vessels on brain surface	Right cerebellar hemisphere
24 M 76 yr	Patient found dead	Firm, well circumscribed tumor attached to dura	Left cerebral hemisphere
25 F New born	Respiratory difficulty and cyanosis, with death occurring 12 hours after parturition	Tangled layer of vessels covering extensive area of brain surface	Medial surface of left cerebral hemisphere
26 F 28 days	Generalized hypotonia, tremor of right upper limb, respiratory difficulty, rapid downhill course	Extensive subarachnoid hemorrhage	Cerebellar surface

hence is not considered of fundamental significance.

There is no characteristic clinical picture for angiomatous meningioma. Convulsive seizures plus various other manifestations may occur with cerebral lesions. Symptoms referable to the cerebellum, the brain stem and the cranial nerves are often observed with predominantly cerebellar lesions. A complicating subarachnoid bleeding may account for many of the clinical features.

*Gross Pathology*—The angiomatous meningioma is observed on the surface of the brain as a plexus of large coiled blood vessels. Serial sections through the involved regions demon-

87 Worster-Drought, C, and Ballance, C A. *Lancet* 2 125, 1922



state that the underlying parenchyma is not involved, thereby differentiating this vascular tumor from the deeper racemose angioma, which primarily involves the brain tissue and extends outward to reach the meninges. The angiomatous vessels may show a loose arrangement or appear as a dense vascular plexus completely hiding the underlying cortex (case 25). Large vascular channels often drain into the dural sinuses. The meningeal angioma may be confined to one region of the brain or may cover extensive areas of the cerebral surface. It may be found over the surface of the cerebrum, the cerebellum or the brain stem. In the cases tabulated here the cerebellum was the favorite location (cases 21, 22, 23 and 26).

A subarachnoid hemorrhage resulting from a rupture of one of the angiomatous vessels is a common complication (cases 21, 22, 23 and 26). This bleeding may be extensive and may so obscure the vascular tumor that the latter may be readily overlooked on gross examination (case 21). When such a subarachnoid extravasation occurs in the cerebellar region, it generally produces compression of the fourth ventricle and concomitant internal hydrocephalus.

A well circumscribed firm tumor of the meninges comprised of closely packed small blood vessels is an extremely rare form of angiomatous meningioma (case 24). Another rare variety is composed entirely of capillaries and is difficult to identify on gross examination (case 26).

*Microscopic Anatomy*—Histologic study confirms the macroscopic observation that angiomatous tumor is confined primarily to the leptomeninges. A few vessels may be scattered along the subpial region of the underlying cortex, but the subcortical parenchyma is not involved. The structure of the vascular elements resembles that of arteries or veins, in other words, the microscopic anatomy is similar to that of venous or arteriovenous angioma except that the individual vessels are separated by loose connective tissue rather than cerebral parenchyma (cases 21, 22 and 23). Vessels of typical arterial structure were prominent in the cases tabulated here. In some instances the lesion is comprised of closely packed cavernous spaces (Globus<sup>8</sup>) or capillaries (case 26), thus simulating cavernoma or telangiectasis.

A rare form of angiomatous meningioma consists of a firm, well circumscribed lesion grossly. Microscopically it is comprised of a large number of small blood vessels situated close to one another (case 24). The walls of these vessels are greatly thickened with various degrees of associated occlusion of the lumens. In all the vessels profound hyaline degeneration is observed, and

in a few extensive mural calcification. In some places the vessels are separated by thin bands resembling meningotheiomatous tissue, suggesting that such a lesion might have a mild transitional tendency toward the meningotheiomatous meningioma.

#### STURGE-WEBER DISEASE

*Introduction*—Sturge-Weber disease consists of atrophy and calcification of the cortex of one cerebral hemisphere, which is generally associated with dilated or actually angiomatous vessels in the overlying meninges and an ipsilateral facial nevus. Even though many of the cases recorded in the literature were published as cases of cerebral angioma, it is questionable whether Sturge-Weber disease should be included with the vascular tumors, since either the vascularity of the meninges or the facial nevus may be absent. The most consistent finding is the calcified, atrophic cortex.

Sturge<sup>88</sup> published one of the first clinical reports in 1879. Later, Weber<sup>89</sup> noted the characteristic roentgenographic findings, which he erroneously ascribed to calcification of an intracranial angioma. Unfortunately, this misconception persisted in many of the subsequent publications and has occasioned much of the confusion in understanding the vascular tumors and malformations. Although the title "Sturge-Weber disease" has appeared frequently in the foreign literature, it has been almost absent from American publications, in which most of the cases are found recorded as cases of angioma. This error probably can be largely attributed to the popularity of the monographic study published by Cushing and Bailey, in which classic cases were regarded as cases of venous angioma. It was not until 1934 that Krabbe<sup>90</sup> proved beyond doubt that the roentgenographic picture was a reflection of the cortical calcification rather than of angioma. Krabbe suggested that the name of this condition be changed to "Kraft-Weber-Dimitri disease" in recognition of Dimitri's<sup>91</sup> beautiful descriptions of the roentgenographic picture. However, since the term "Sturge-Weber disease" has been so widely employed, it is questionable whether such an alteration is feasible.

There are now a large number of instances of Sturge-Weber disease recorded in the literature. As early as 1913, Hebold<sup>92</sup> reported 5 cases. In 1936 a review of the literature revealed 16-

88 Sturge, W. *Tr Clin Soc London* **12** 162, 1879.

89 Weber, F. P. *Arch Neurol & Psychiat* **3** 134, 1922.

90 Krabbe, K. H. *Arch Neurol & Psychiat* **32** 737, 1934.

91 Dimitri, V. *Rev Asoc med argent* **36** 63, 1923.

92 Hebold, O. *Arch f Psychiat* **51** 445, 1913.

ports of 68 examples of this disease (Greenwald and Koota <sup>93</sup>), however, the failure to recognize this condition, with the result that many cases were recorded under a variety of titles, makes it virtually impossible to discover all the recorded cases

The outstanding clinical signs and symptoms consist of convulsive seizures, an ipsilateral facial nevus, contralateral hemiparesis and hemianopsia, ipsilateral exophthalmos, glaucoma and parallel, wavy lines of cortical calcification observed on roentgenograms of the skull

*Gross Pathology*—Few cases have been studied pathologically, and no material has been available to me for study In the recorded cases the cerebrum showed atrophy involving only an isolated area or an entire hemisphere There was a rather definite predilection for the occipital region, however, the case tabulated here was an exception since air studies revealed atrophy which spared only the occipital lobe The atrophic areas appear shrunken, and the convolutions become

TABLE 8—A Case of Sturge-Weber Disease

Case, Sex, Age	Symptoms and Findings	Roentgenologic Features
27 F 31	Port wine nevus of right side of face, left hemiparesis and hemihypesthesia, optic atrophy, convulsive seizures	Roentgenograms of skull and encephalography revealed atrophy of almost the entire right cerebral hemisphere, with wavy lines representing calcification of the cortical gray matter

narrow, hypoplastic and firm in consistency Cut sections disclose marked diminution of the cerebral tissues eventuating in secondary dilatation of the regional ventricle There is notable thinning of the cortical ribbon, in which macroscopic calcification is often visible Occasionally large irregular nodules of calcification are observed (Krabbe <sup>90</sup>) Associated cerebellar atrophy is a rare concomitant occurrence (Hebold <sup>92</sup>)

The surface of the atrophic cerebrum is often covered with a congeries of enlarged angiomatous vessels or an actual angiomatous meningioma In many cases, however, only a few dilated vessels are revealed, in a few cases there are no signs of any abnormal vascularity (Krabbe <sup>90</sup>, Kreyenberg <sup>40</sup>, Gerhard and Hansing <sup>40</sup>, Geylin and Penfield <sup>94</sup>) Ipsilateral buphthalmos or exophthalmos is a common finding Also large dilated vessels may occur in the sclera (case 31)

*Microscopic Anatomy*—The involved regions reveal a thin atrophic cortex in which there is

relatively complete absence of neurons and axons In addition, there is a prominent increase of fibrous astrocytes forming a dense interlacing meshwork of glial fibers The small cortical blood vessels show extreme thickening and hyalinization of their walls and resultant narrowing of their lumens Many of these vessels are completely calcified, with small calcific deposits situated in the intervacular tissue

When an angioma is present, it is usually confined to the overlying leptomeninges It is unusual for the angioma to invade the underlying cortex, however such an occurrence was reported by Hasson <sup>40</sup> The structure of the angiomatous vessels generally resembles that of normal arteries or veins Calcification of these vessels is not at all striking Small heterotopias of cerebral tissue have been encountered in the leptomeninges by Bergstrand, Olivecrona and Tonnis <sup>3</sup>

A vascular involvement usually resembling cavernous angioma may be found in the retina and the sclera of the diseased eye (Meller <sup>95</sup>), but others claim there may be no pathologic change to explain the ocular manifestations (Weber <sup>89</sup>)

The genesis of the cortical atrophy remains a moot point While certain authors have considered this condition secondary to circulatory disturbances occasioned by the overlying angioma, others have regarded it as congenital aplasia of the cortex The frequent occurrence of other congenital anomalies tends to favor the latter point of view

ANGIOBLASTOMA

*Introduction*—Angioblastoma is comprised of embryonic vascular elements and constitutes one of the most common intracranial vascular lesions The tumor often occurs as a nodule in the wall of a cerebellar cyst Because of the close macroscopic resemblance to cerebellar astrocytoma, these two lesions usually were not distinguished in the earlier publications However, after the appearance of Lindau's <sup>2</sup> original article in 1926, angioblastoma has been widely recognized, with the result that a relatively abundant literature is now available

The terms used, especially in the earlier records of cases, are most confusing For some time tumors of this type were regarded as glioma (Roussy, Lhermitte and Cornil <sup>96</sup>, Cushing and Bailey <sup>1</sup>, Bassoe and Apfelbach <sup>97</sup>) Other

93 Greenwald, H M, and Koota, J Am J Dis Child 51 868, 1936  
94 Geylin, H R, and Penfield, W Arch Neurol & Psychiat 21 1020, 1929

95 Meller, J Arch f Ophth 85 255, 1913  
96 Roussy, G, Lhermitte, J, and Cornil, L Ann d'anat path 1 333, 1924  
97 Bassoe, P, and Apfelbach, C W Arch Neurol & Psychiat 14 396, 1925

angioblastic lesions were referred to as cystic sarcoma (Cushing and Bailey<sup>1</sup>), sarcomatous hemangioma (Corten<sup>98</sup>), hemangiomatous cyst (Sargent and Greenfield<sup>99</sup>) and angioreticuloma (Roussy and Oberling<sup>100</sup>, Bergstrand, Olivecrona and Tonnis<sup>3</sup>). Not infrequently, even in recent articles, one encounters names of entirely different vascular lesions used for these neoplasms as, for example, "telangiectasis," "capillary hemangioma" or "angioma." Out of respect for Lindau's<sup>2</sup> pioneering studies, the lesion occasionally is spoken of as Lindau's tumor. Similar angioblastic tumors in other parts of the body generally are called hemangioendothelioma, a name which only occasionally is employed for the cerebellar tumors.

superfluous since lymphatic neoplasms of the nervous system are unknown. The term "angio-reticuloma" is in keeping with the abundant reticulum of these tumors but is less widely known than the title "angioblastoma."

The clinical picture differs little from that of other cerebellar tumors. Treatment consists of early surgical removal of the mural nodule. Such a procedure frequently results in complete recovery of the patient with no recurrence of the tumor if it has been completely excised.

*Gross Appearance*—Cerebellar angioblastoma has been described from the standpoint of pathology by a number of writers. A few of these are Joseph<sup>102</sup>, Friedrich and Stiehler<sup>103</sup>, Bassoe and Apfelbach<sup>97</sup>, Lindau<sup>2</sup>, Cushing and

TABLE 9—Cases of Angioblastoma Studied in the Departments of Neuropsychiatry and Pathology of the University of Minnesota

Case	Sex	Age, Yr	Symptoms and Findings	Gross Appearance	Location
28	M	40	Suboccipital cephalalgia, cervical rigidity, symptoms of increased intracranial pressure, slight paresis of left upper extremity, symmetric hydrocephalus on ventriculography	Mural nodule in a large cyst	Left cerebellar hemisphere
29	M	30	Deafness of right ear, spells of unconsciousness, occipital and nuchal pain, papilledema, anisocoria, paresthesias of left side of body, right hemihypesthesia, scattered irregularities of reflexes	Internal hydrocephalus, soft red tumor containing small cysts, well circumscribed	Upper part of medulla
30	F	38	Evidence of intracranial hypertension, occipital headache and tenderness, left exophthalmos, paresthesias of ulnar border of left hand	Solid reddish tumor nodule 2.4 cm in diameter, large engorged vessels in involved region, internal hydrocephalus	Region of right cerebellar tonsil
31	F	12	Occipital headache, cervical rigidity, signs of increased intracranial pressure, anisocoria, tonic fits, dysmetria in right upper limb, positive toe signs	Firm red nodule 2 cm in diameter in cyst wall	Anterosuperior part of right cerebellar hemisphere
32	M	45	Occipital cephalalgia, increased intracranial pressure, epigastric pain, ataxia with right	Reddish cystic tumor mass	Region of cisterna magnum and lower fourth ventricle
33	M	28	Fronto-occipital headaches, advanced picture of increased intracranial pressure, ataxia, scattered changes in reflexes	Small reddish tumor nodule attached to cyst wall	Right cerebellar hemisphere (left hemisphere compressed)
34	F	25	Onset during pregnancy. Suboccipital pain, symptoms of elevated intracranial pressure, deafness of right ear, hyperactive reflexes, incoordination on right ataxia	Grayish red neoplastic nodule in wall of a cyst	Left cerebellar hemisphere
35	M	23	Right sided occipital headaches, choked disks with other signs of increased pressure, skew deviation of eyes, slurred speech, marked right sided cerebellar manifestations	Small cyst containing a mural tumor nodule	Right cerebellar hemisphere

Cushing and Bailey<sup>1</sup> coined the term "angioblastoma." They noted that morphologically this neoplasm closely resembled the various stages that the vasoformative cells pass through during the early development of the vascular system as demonstrated by Sabin<sup>101</sup> in hanging drop preparations of the chick embryo. This observation gave the title "angioblastoma" a structural connotation, which most likely accounts for its universal acceptance. The prefix of the name "hemangioblastoma," which is often used, seems

Bailey<sup>1</sup>, Greenfield<sup>104</sup>, Bergstrand and his co-workers<sup>3</sup>, Levine<sup>5</sup>. Although angioblastoma may involve any region of the central nervous system, it shows a predilection for the hemispheres of the cerebellum (cases 28, 31, 33, 34 and 35). Another relatively common situation is the posterior portion of the cerebellar vermis (Cushing and Bailey<sup>1</sup>, present report, case 33). Occasionally, it is encountered in the brain stem as in case 29 and in cases reported by Berblinger,<sup>105</sup> Tannenberg,<sup>106</sup> Lindau<sup>2</sup> and many

<sup>98</sup> Corten, M. H. Frankfurt Ztschr f Path **29** 693, 1921

<sup>99</sup> Sargent, P., and Greenfield, J. G. Brit J Surg **17** 84, 1929

<sup>100</sup> Roussy, G., and Oberling, C. Presse méd **38** 179, 1930

<sup>101</sup> Sabin, F. R. Anat Rec **13** 199, 1917

<sup>102</sup> Joseph, M. Ztschr f klin Med **16** 347, 1889

<sup>103</sup> Friedrich, H., and Stiehler, H. Deutsche Ztschr f Nerven **73** 158, 1922

<sup>104</sup> Greenfield, J. G. Proc Roy Soc Med **24** 382, 1931

<sup>105</sup> Berblinger, W. Arch f Ophth **110** 395, 1922

<sup>106</sup> Tannenberg, J. Ztschr f d ges Neurol u Psychiat **92** 119, 1924

others. In a few instances of angioblastoma of the brain stem or spinal cord, concomitant syringomyelia has been noted by Lindau,<sup>2</sup> Gaupp,<sup>107</sup> Pinnet,<sup>108</sup> Schuback,<sup>109</sup> and others. Angioblastoma of the cerebrum is a rare pathologic curiosity, but a few acceptable cases have been reported (Rochat<sup>110</sup>, Bielschowsky<sup>111</sup>, Berger and Guleke<sup>112</sup>, Schley<sup>113</sup>, Mariotti<sup>114</sup>). In many of the cases of intracerebral angioblastoma the lesion involves the regional meninges and hence may actually be meningioma (Barnard and Walshe<sup>115</sup>, Wolf and Cowen<sup>116</sup>, Keller<sup>117</sup>). Although more than one lesion is not common, multiple angioblastoma of the nervous system occasionally occurs, according to Koch,<sup>118</sup> Wersilow,<sup>119</sup> Schuback,<sup>109</sup> Moller,<sup>120</sup> Guillain and co-workers,<sup>121</sup> Davidson and co-workers,<sup>122</sup> Raney and Courville<sup>4</sup> and others.

The involved cerebellar hemisphere is often enlarged, compressing the brain stem and at times the opposite cerebellar hemisphere. The cerebellar tonsil may be forced through the foramen magnum. Aspiration of the involved fluctuating area generally yields a clear straw-colored fluid, which may quickly coagulate. Midline lesions are easily detected from the surface, since they spread the cerebellar tonsils. Enlarged, dilated blood vessels may be observed over the involved cerebellar surface. The fourth ventricle is always compressed, a condition eventuating in marked internal hydrocephalus. The floor of the fourth ventricle, however, is only rarely invaded (Zeitlin<sup>123</sup>).

107 Gaupp, J. Beitr. z. path. Anat. u. z. allg. Path. **2**: 510, 1888.

108 Pinnet, A. W. Arb. a. d. path. Anat. Inst. zu Tübingen **9**: 118, 1914.

109 Schuback, A. Ztschr. f. d. ges. Neurol. u. Psychiat. **110**: 359, 1927.

110 Rochat, G. F. Nederl. tijdschr. v. geneesk. **1**: 1124, 1927.

111 Bielschowsky, M. Deutsche Ztschr. f. Nervenheilk. **22**: 54, 1902.

112 Berger, H., and Guleke. Deutsche Ztschr. f. Chirurg. **203-204**: 104, 1927.

113 Schley, W. Centralbl. f. allg. Path. u. path. Anat. **41**: 337, 1928.

114 Mariotti, D. Pathologica **28**: 1, 1936.

115 Barnard, W. G., and Walshe, F. M. R. J. Path. & Bact. **34**: 385, 1931.

116 Wolf, A., and Cowen, D. Bull. Neurol. Inst. New York **5**: 485, 1936.

117 Keller, H. Virchows Arch. f. path. Anat. **289**: 151, 1933.

118 Koch, F. Ztschr. f. Kinderheilk. **59**: 638, 1938.

119 Wersilow, W. Neurol. Centralbl. **32**: 340, 1913.

120 Moller, H. V. Acta ophth. **7**: 244, 1929.

121 Guillain, G., Schmide, P., and Butrand, I. Rev. Neurol. **1**: 420, 1932.

122 Davidson, C., Brock, S., and Dyke, C. G. Bull. Neurol. Inst. New York **5**: 72, 1936.

123 Zeitlin, H. J. Neuropath. & Exper. Neurol. **1**: 14, 1942.

Cut sections of the cerebellum, as a general rule, reveal a large part of the hemisphere to be replaced by a large cyst containing clear yellow fluid. The walls of the cyst are smooth, showing no tumor except at the site of attachment of the mural nodule, which comprises the entire neoplasm. The tumor is either sessile or pedunculated, measuring only about 1.5 to 2.5 cm. in diameter. It is grayish to dark red and firm in consistency.

A less common form of angioblastoma consists of a solid, fairly well circumscribed tumor which is not associated with a large cyst (case 33). It generally contains multiple small cysts, which may be visible grossly (cases 29 and 32). In the rare instances in which angioblastoma occurs in the brain stem it may be solid or cystic. Cushing and Bailey's<sup>1</sup> division of the angioblastic lesions into cystic, partially cystic and solid varieties has little value since there are all sorts of transitional variations in the cystic structure.

*Microscopic Anatomy*—Except for minor variations, the histologic structure of angioblastoma is remarkably constant. Small capillaries and occasionally a few cavernous spaces are disseminated throughout the tumor. These vessels are generally filled with blood. The intervascular areas are cellular and contain numerous tiny embryonic vascular spaces, which are usually empty (fig. 3). These tiny spaces are lined by either endothelial or actual neoplastic cells. The cells of the intervascular areas merge directly with those lining the vascular channels and appear to be growing from the outer surface of the capillary walls.

The typical neoplastic cell has an oval vesicular nucleus and often a nucleolus. The cytoplasm generally contains a few vacuoles. Sometimes the cytoplasm is extensive and filled with large vacuoles, giving the cell a foamy appearance. Cells of this type are referred to as pseudo-xanthomatous or xanthic cells because of their resemblance to the foam cells characteristic of xanthoma (Lindau<sup>2</sup>, Turner and Kernohan<sup>13</sup>). Polariscopic studies suggest that the lipid contained in these vacuoles is closely related to a cholesterol ester (Zeitlin<sup>1-3</sup>). It is most likely nutritional rather than degenerative in origin since it shows no constant relationship to areas of degeneration. Large foam cells were prominent in 4 of the cases tabulated here (28, 29, 31 and 32). In some areas of the tumor the nuclei may become pyknotic or appear elongated and fusiform. Multinucleated giant cells and large cells containing bizarre-shaped nuclei occasionally are observed.

Mitotic figures are seldom seen. A dense reticulum permeating the tumor is characteristic of these lesions and is best demonstrated by the Perdrau technic. The reticulum fibers encircle the capillaries and vascular spaces, sending branches into the cellular intervacular areas. Occasionally the reticulum is missing in the highly cellular areas. In certain regions of the tumor capillaries may be extremely prominent, while in other areas the structure is extremely cellular with the vascular nature being indicated merely by the tiny embryonic spaces. Lesions in which cavernous spaces are predominant may be confused with cavernous angioma. However, the cavernous walls are more delicate than those of true cavernoma, and proliferating cells as well as embryonic vascular spaces are observed between the cavernous vessels. Also, areas of typical angioblastic structure are usually found in some portions of the tumor, proving conclusively that the lesion is angioblastoma rather than cavernoma.

Occasionally the neoplastic structure of certain regions of angioblastoma is distorted or even obliterated by a proliferation of fibrous connective tissue or fibrillary neuroglia (cases 28, 29, 33 and 34). The resultant morphologic alterations were termed sclerosing changes by Bailey and Ford<sup>24</sup>. Areas of necrosis (case 35), cystic cavities, edematous regions, collections of blood pigment or a marked proliferation of the endothelial lining of the larger capillaries (case 35) may sometimes be observed in angioblastoma.

The tumor is well circumscribed and often is circumvented by a zone of fibrous neuroglia (case 29). The wall of the cyst consists of glial fibers and contains no tumor tissue except at the site of attachment of the mural nodule. The cystic fluid is considered to be the result of transudation and resembles plasma in its chemical composition (Martin). The fluid quickly reforms after aspiration if the tumor nodule is not removed.

#### LINDAU'S (VON HIPPEL'S) DISEASE

*Introduction*—This condition consists of an angioblastic tumor of the retina associated with single or multiple angioblastoma of the nervous system and various congenital lesions of other organs, such as cystic disease of the kidneys, the pancreas and the liver. Von Hippel's disease refers to an isolated angioblastic lesion of the retina, while the entire complex is termed Lindau's disease after the author who first popularized knowledge of the condition. Lindau<sup>2</sup> found that in 20 per cent of the recorded cases of von Hippel's disease there was evidence of

associated neural lesions. Lindau's disease also has been called von Hippel-Lindau disease (Davidson, Brock and Dyke<sup>122</sup>) and Lindau-von Hippel disease (Craig, Wagener and Kernohan<sup>124</sup>).

The retinal lesion was first described by Fuchs<sup>125</sup> and Wood,<sup>126</sup> but Collins<sup>127</sup> was the first to recognize its true angioblastic structure. Although von Hippel<sup>128</sup> did not report this condition of the retina until 1903 and erroneously regarded it as an arteriovenous aneurysm, the disease still bears his name. Lindau's disease was first described by Schuback<sup>100</sup> but was not widely recognized until after Lindau's monograph. Cushing and Bailey<sup>1</sup> pointed out that Lindau overstressed the frequency of this condition and demonstrated that angioblastoma occurs much more often as an isolated cerebellar tumor. In the 8 cases of angioblastoma tabulated here there was no vascular tumor of the retina, however, in case 34 an associated cystic disease of the pancreas was found.

The clinical picture is most variable, being characterized by ocular and nervous system manifestations. Either a family history of the disease, which is obtained in about 20 per cent of the cases, or visualization of the retinal lesion is necessary for an antemortem diagnosis.

*Pathologic Features*—A small tumor nodule which reveals angioblastic structure is found in the peripheral portion of the retina. A large tortuous artery and vein connect the neoplastic nodule with the optic disk. An associated cyst similar to that of the cerebellar lesion may produce a detachment of the retina. Secondary changes such as proliferation of connective tissue, organization of extravasated blood, adherence of the retina to the lens and calcification of the choroid may occur. These changes may obscure the vascular nature of the lesion and have been referred to as Coats's retinitis (Coats<sup>129</sup>). In contrast to the angioblastoma of the nervous system, the retinal neoplasm may undergo calcification and occasionally even ossification (Davidson, Brock and Dyke<sup>122</sup>).

By definition, single or multiple angioblastoma of the nervous system is present. The lesion of the nerve tissues possesses the same gross and microscopic features as the isolated lesion described in the previous section. Angioblastoma

124 Craig, W. M., Wagener, H. P., and Kernohan, J. W. *Arch Neurol & Psychiat* **46** 36, 1941.

125 Fuchs, E. *Arch f Augenh* **11** 440, 1882.

126 Wood, D. J. *Tr Ophth Soc U Kingdom* **12**: 143, 1892.

127 Collins, E. T. *Tr Ophth Soc U Kingdom* **14** 141, 1894.

128 von Hippel, E. *Arch f Ophth* **59** 83, 1904.

129 Coats, G. *Arch f Ophth* **81** 275, 1912.

of the cerebellum is the most common but involvement of the brain stem and the spinal cord is not infrequent Syringomyelia, hydromyelia or syringobulbia occasionally is found as a concomitant lesion, according to Davidson and his associates,<sup>122</sup> Craig and his associates<sup>124</sup> and others

Cystic disease of the kidneys, the pancreas and the liver, as well as hypernephroma, is common The hypernephroma does not metastasize Other visceral lesions, such as an adenoma of the epididymis, a cavernoma of the liver (Koch<sup>130</sup>), an angioma of the kidney or a small cerebellar ependymoma, may occur (Craig and his co-workers<sup>124</sup>, Kernohan, Woltman and Adson<sup>131</sup>)

Although the cystic disease of the various organs has been regarded as very pathognomonic of this condition, such involvement of organs may occur in the absence of angioblastoma of the retina and the nervous system Therefore, the angioblastic lesions are essential for a diagnosis of Lindau's syndrome

ANGIOBLASTIC MENINGIOMA

*Introduction*—Angioblastic meningioma is a rare form of meningeal tumor comprised of capillaries, embryonic vascular elements and an abundant stroma of reticulum The term "angioblastic meningioma" was introduced by Bailey, Cushing and Eisenhardt<sup>172</sup> They used this term interchangeably with "supratentorial angioblastoma" since their meningeal vascular tumors showed a predilection for the superior surface of the tentorium More recently, however, the latter term has been used for the intracerebral as well as the meningeal angioblastic lesions A few other titles occasionally have appeared in the literature such as "leptomeningioma piale" (Globus<sup>8</sup>), "malignant meningioma" (Bergstrand and his co-workers<sup>3</sup>), "capillary angioma" (Keller<sup>117</sup>), "perithelioma" (Borst<sup>133</sup>), "hem-angioendothelioma" (Bruns<sup>134</sup>), "angiosarcoma" (Ribbert<sup>17</sup>) and "endothelioma angiomatosum" (Henschen<sup>135</sup>)

Since this variety of meningioma is rather rare and has been adequately described only in recent years, the literature regarding this lesion is relatively meager Carefully verified cases have been published by Wolf and Cowen,<sup>116</sup> Tannenbergs,<sup>106</sup> Bailey and Bucy,<sup>136</sup> Bailey, Cushing and Eisenhardt,<sup>132</sup> Cotten,<sup>98</sup> Zeithin,<sup>123</sup> Globus,<sup>8</sup> Bergstrand and his co-workers,<sup>3</sup> Bailey and Ford,<sup>21</sup> Courville and Abbot<sup>26</sup> and Patterson and Anderson<sup>137</sup>

The clinical picture differs little from that of any other type of meningioma and depends largely on the location of the lesion The clinical course is often rapid but may be prolonged Although complete surgical removal is possible, postoperative recurrence is the rule

*Gross Appearance*—On gross examination alone, angioblastic meningioma cannot be differentiated from the more common varieties of

TABLE 10—Cases of Angioblastic Meningioma Studied in the Departments of Neuropsychiatry and Pathology of the University of Minnesota

Case, Sex, Age	Symptoms and Findings	Gross Anatomy	Location
36 M 46 yr	Increased intracranial pressure, right homonymous hemianopsia, spastic paresis of right lower limb, right hemihypesthesia	Firm fleshy meningeal tumor 5 cm in diameter grossly infiltrating adjacent cerebral cortex	Left temporal lobe
37 F 56 yr	Headache, right hemiparesis and hemihypesthesia	Fairly well circumscribed meningeal tumor 5 cm in diameter adherent to underlying cerebral cortex	Rolandic region of left cerebral hemisphere
38 F 62 yr	Personality changes, headache, confusion, hyperactive reflexes, bilateral positive Babinski signs	Firm, well circumscribed globoid tumor 7 by 8 cm in diameter adherent to dura	Orbital surface of right frontal lobe

meningioma Generally the outer surface is firmly adherent to the dura but only in rare instances does the growth invade the overlying bone (Zeithin<sup>123</sup>) The tumor usually assumes a globoid form but at times appears as a flat, plaque-like lesion Although grossly the tumor appears to be well circumscribed, invasion of the adjacent parenchyma often occurs (cases 36 and 37) In most instances the tumor is firm or very fibrous in consistency The cut surface varies from reddish brown to yellowish white (cases 36 and 37) Although gross examination may show striking vascularity, some of the more common forms of meningioma also possess copious vascularity

Apparently any region of the brain may be involved by this type of meningioma However,

136 Bailey, P, and Bucy, P C Am J Cancer 15 15, 1931  
137 Patterson, G H, and Anderson, F M Bull Los Angeles Neurol Soc 5 218, 1940

130 Koch, K Virchows Arch f path Anat 214 180, 1913  
131 Kernohan, J W, Woltman, H W, and Adson, A W Arch Neurol & Psychiat 25 679 1931  
132 Bailey, P, Cushing, H, and Eisenhardt, R Arch Path 6 953, 1928  
133 Borst, M Echte Geschwulste, in Aschoff, L Pathologische Anatomie, Jena, G Fischer, 1913, p 497  
134 Bruns, L Die Geschwulste des Nervensystems Hirngeschwulste, Rückenmarks und Wirbelgeschwulste Geschwulste der peripheren Nerven, Berlin, S Karger, 1908, p 19  
135 Henschen, F Ueber Geschwulste der hinteren Schadelgrube, insbesondere des Kleinhirn-brückenwinkels, Jena, Gustav Fischer, 1910, p 84



the cases described in the literature as well as those tabulated here indicate that there is definite predilection for the cerebral convexities. The superior surface of the tentorium or the peritumoral region ranks next in frequency of involvement. The tumor also may be found in other locations, such as the regions of the inferior surface of the tentorium, the cerebellum, the olfactory groove (case 38), the cerebellopontile angle and the midline between the cerebral hemispheres, where it is adherent to the falx.

The large globoid form occasions a notable compression of the neighboring regions of the brain (case 38). Generally speaking, angioblastic meningioma is more invasive than the more frequently occurring, slower growing meningeal tumors.

*Microscopic Anatomy*—The morphology of angioblastic meningioma is similar to that of cerebellar angioblastoma. In occasional cases the microscopic structure may be identical (Bailey, Cushing and Eisenhardt<sup>132</sup>). The meningeal tumor is composed of capillaries, cavernous spaces, proliferating endothelial cells or angioblasts and embryonic vessels lined by neoplastic cells. The cytoplasm of the tumor cells may contain a few vacuoles, but the characteristic large pseudoxanthomatous cells are not prominent. Not infrequently, the angioblastic cells tend to become elongated and spindle shaped throughout extensive portions of the tumor. Evidence of rapid growth is usually present as demonstrated by numerous mitotic figures (cases 36, 37 and 38). A few examples exhibited a striking variation in the size and the shape of the tumor cells as well as numerous giant forms containing large bizarre hyperchromatic nuclei. Wolf and Cowen,<sup>116</sup> Courville and Abbott<sup>26</sup> and others regarded this cellular variation to be indicative of high malignancy.

Other variations in the morphologic structure may occur. Scattered areas may closely resemble mesenchymal tissue (case 36). Also there may be a tendency to whorl formation, often with production of psammoma bodies, suggesting that the lesion is true meningioma (Bergstrand and Olivecrona<sup>138</sup>), however, these alterations might be attributed to independent inclusions of arachnoid (Wolf and Cowen<sup>116</sup>). Transitional lesions containing extensive areas of meningotheomatous structure have been described by Cushing and Eisenhardt<sup>25</sup> and Courville and Abbott<sup>26</sup>. When considering these transitional neoplasms, however, one must be cautious since a slight

angioblastic tendency is not infrequent in the more common types of meningioma.

Many of the variations in the microscopic structure of this tumor type may be the result of proliferation of the stromal elements. Bailey and Ford<sup>21</sup> referred to this alteration as a "sclerosing change." They felt that this sclerosing process accounted for the structural differences between the meningeal and the cerebellar angioblastoma.

Perdrau preparations demonstrate an abundance of reticulum throughout this tumor. The reticulum outlines the vessels and sends a network of branching processes into the intervascular regions. In those areas comprised of elongated fusiform cells the intervascular branches may be absent, with reticulum being observed only around the vascular channels (case 37).

#### ANGIOSARCOMA

The term "angiosarcoma" is occasionally used to describe vascular tumors revealing histologic evidence of rapid growth (Ribbert<sup>139</sup>, Turner and Kernohan<sup>13</sup>). In a large number of cases primary sarcoma of the brain is highly vascular, with collections of tumor cells encircling the blood vessels. Such a structure has suggested the titles "perivascular sarcoma," "plexiform angiosarcoma" and "perithelial sarcoma" to Borst<sup>133</sup> and others. This high degree of vascularity led Globus<sup>8</sup> to regard all the sarcomatous tumors as vascular lesions belonging to the group of neoplasms he entitled leptomeningioma piale. However, in many cases primary sarcoma of the nervous system fails to show angioblastic activity with resultant embryonic vascular channels, therefore, it should not be considered to be a vascular neoplasm. The term "angiosarcoma" applies only to a rapidly growing mesodermal tumor of the nervous system presenting angioblastic structure.

In general, an angioblastic tumor showing sufficient characteristics to be considered as angiosarcoma occurs as a meningeal rather than as an intracerebral or a cerebellar lesion (cases 36, 37 and 38). Hence, if such a term were accepted, it would include essentially the entire group of tumors classifiable as angioblastic meningioma. However, a few tumors of the latter group which are free from any anatomic evidence of rapid growth would be excluded from the group described as vascular sarcoma. Thus, even though the angiosarcoma group would consist entirely of tumors classifiable as angioblastic meningioma, not all of the tumors classed as

<sup>138</sup> Bergstrand, H., and Olivecrona, H. *Am J Cancer* 24: 522, 1930.

<sup>139</sup> Ribbert, H. *Geschwulstlehre für Aerzte und Studierende*, Bonn, Friedrich Cohen, 1904, p. 591.



meningeal angioblastoma would be included. In addition, structures on the borderline of cancer would be difficult to classify. If in the future angioblastic tumors of rapid growth are found within the cerebral parenchyma, they should be classified as angiosarcoma.

#### ANGIOGLIOMA

*Introduction*—The term “angioglioma” was introduced by Roussy and Obeiling<sup>100</sup> in 1930 to designate a tumor comprised of adult vascular and glial elements. These authors expressed the opinion that this lesion was a neoplastic entity representing a transition between angioma and glioma. Only a few cases have been recorded in the literature, since this variety of tumor has not been generally accepted. Of the more than 700 cerebral tumors histologically verified in the departments of neuropsychiatry and pathology of the University of Minnesota, only 1 revealed structure suggestive of this type of lesion (table 11).

TABLE 11—4 Case of Angioglioma

Case, Sex, Age	Symptoms and Findings	Gross Appearance	Location
39 M 25 yr	Head injury, convulsive seizures, headache, intellectual failure, hallucinations, increased intracranial pressure and roentgenographic evidence of calcification in right frontal region	A diffusely infiltrating firm and rubbery tumor containing disseminated foci of calcification	Right frontal lobe

There are no diagnostic clinical features. The picture is that of a slowly growing tumor of the brain, the resultant symptoms and findings being appropriate for the region involved by the lesion.

*Gross Pathology*—Angioglioma may occur as a nodular tumor attached to the wall of a cyst or as a poorly circumscribed solid type of lesion. The cystic variety presents an appearance resembling astrocytoma except that it more frequently contains grossly visible areas of calcification (Bergstrand and his co-workers<sup>3</sup>). The solid variety is observed as a diffusely infiltrative lesion similar in appearance to astrocytoma. The cerebral structures involved by this noncystic tumor are firm and rubbery and exhibit small focal deposits of calcium (case 39). While any region may be involved by angioglioma, it most commonly is found in the cerebellum, where it occurs as a cystic tumor. In the cerebral hemispheres it generally assumes the appearance of a diffusely invasive lesion. Rare cases of multiple angioglioma have been reported (Wolff and Donat<sup>140</sup>).

*Microscopic Anatomy*—Histologically, angioglioma is composed of angiomatous and glial structures. The glial elements as a rule are represented by fibrous astrocytes which commonly have produced heavy glial fibers (case 39). At times there may occur an admixture of astrocytes and oligodendroglia. In a few instances the glial component assumes the structure of spongioblastoma polare (Bergstrand and his associates<sup>3</sup>). In all cases small blood vessels representing the vascular component are abundantly disseminated throughout the neoplasm. The vessel walls are often thickened and infiltrated by calcific granules. The noncalcified vessels possess thick hyalinized walls containing neither smooth muscle nor elastic tissue. These mural alterations occasion various degrees of luminal attenuation, including complete occlusion.

This transitional form of tumor has been accepted as a nosologic entity by only a few authors and has been rejected by many. Bailey and Ford<sup>24</sup> suggested that such a structure eventuated from the replacement of the original cellular elements in angioblastoma by proliferation of its glial stroma. Turner and Kernohan,<sup>13</sup> however, preferred to regard it as angioma associated with a profound secondary glial reaction. It has also been suggested that this neoplasm merely represents a highly vascular form of glioma. However, if the vascular components are primary rather than secondary, angioglioma may well represent an entity. The occurrence of the typical anatomic structure of astrocytoma without any associated increase in vascularity in many areas throughout the tumor in the case tabulated here (39) suggests that angioglioma may represent merely a variation of astrocytoma rather than a distinct transitional form of neoplasm. Although the validity of the term “angioglioma” is questionable, I feel that the tumor which it designates should be included in studies of the vascular tumors until more material has been studied.

#### COMMENT

In a survey of the literature concerning the intracranial vascular tumors there was encountered a striking diversity of opinion regarding these lesions. The conflicting nomenclature made an accurate identification of the discussed lesion difficult. However, from this mass of conflicting opinions and titles, there has emerged a relatively clear concept of a few fundamental vascular processes, namely, those named angioblastoma and telangiectasis. Many authors have attempted to establish a simple classification of the vascular lesions by dividing them into angioblastoma, telangiectasis and angioma. The last

<sup>140</sup> Wolff, K., and Donat, R. *Ztschr f urol Chir* 43: 272, 1937.

was supposed to be comprised of vessels of an adult type, but the details of its structural variations were poorly understood since it was studied mainly from the clinical manifestations. Descriptions of lesions with this designation were generally limited to what might be noted in a superficial inspection at operation since pathologic studies were rarely made. In numerous cases, therefore, it was impossible to determine whether a lesion such as a racemose angioma involved only the surface of the brain or extended deeply into the cerebral substance. As a result of this paucity of anatomic studies, many different types of vascular lesions were confused, for example, Sturge-Weber disease was regarded in some cases as venous angioma. In addition, a large variety of vascular lesions were omitted from supposedly complete discussions on this subject. The large number of widely conflicting classifications of the vascular tumors evidence the confusion in the published literature.

A careful morphologic study of the structure of the lesions in the cases tabulated in the foregoing pages permitted their being arranged into various groups according to their structure. The lesions in each of these groups possessed a definite type of structure, different from that of any of the other groups. Thus, each variety constituted a pathologic entity. In selecting the title for each type of lesion, a term consistent with the vascular structure as well as with the terms most widely used in the literature was favored.

Before attempting a classification of the vascular tumors, it might be well to list the various forms of these lesions and indicate briefly the groups and the microscopic features which characterize them and which, in my opinion, make them separate and independent entities.

#### I Telangiectasis

- 1 A collection of dilated capillaries
- 2 The capillaries are separated by normal brain tissue

#### II Rendu-Osler disease

- 1 Multiple telangiectasis of the skin, the mucous membranes and various internal organs
- 2 Only occasionally it involves the nervous system

#### III Varix or varicosity

- 1 One or several relatively large dilated vessels of regular contour
- 2 The vessels contain thin walls comprised of an endothelial lining and a layer of hyalinized connective tissue
- 3 Cerebral tissue separates the varicose vessels
- 4 The varix may be situated within the brain or the meninges
- 5 It may rupture to produce intracerebral hemorrhage

#### IV Sinus pericranii

- 1 A midline vascular tumor of the scalp or the forehead
- 2 It communicates with the superior sagittal sinus
- 3 Occasionally there may be an associated intracranial angiomatous lesion

#### V Cavernous angioma or cavernoma

- 1 Closely packed cavernous spaces, situated adjacent to one another without intervening neural tissue
- 2 The cavernous walls are thin, being composed of an endothelial lining and a layer of hyaline connective tissue

#### VI Venous (racemose) angioma

- 1 A congeries of entangled vessels often extending from the brain surface into the underlying parenchyma
- 2 The mural structure of most of the vessels resembles that of veins
- 3 The vessels are separated by degenerated parenchyma

#### VII Arteriovenous (racemose) angioma

- 1 Similar to venous angioma except that vessels resembling arteries are prominent
- 2 The walls of the arterial vessels are composed of a fairly well developed muscular media and an internal elastic membrane

#### VIII Angiomatous meningioma

- 1 A plexus of enlarged vessels restricted principally to the leptomeninges
- 2 The vessels consist of arteries, veins, cavernous spaces or capillaries

#### IX Sturge-Weber disease

- 1 Atrophy and calcification of the cortex of a part or of all of one cerebral hemisphere
- 2 The involved cortex is often covered with an angiomatous meningioma
- 3 There may be a homolateral port wine nevus of the face
- 4 The atrophic cortex shows parenchymal devastation, glial repair and vascular calcification (no angioma)

#### X Angioblastoma

- 1 It occurs most commonly in the cerebellum
- 2 It is often cystic (a nodule in a cyst wall)
- 3 Structurally it consists of numerous capillaries separated by proliferating cells, producing embryonic vascular spaces
- 4 There is an interlacing network of reticulum throughout the tumor

#### XI von Hippel-Lindau disease

- 1 Angioblastoma of both the retina and the nervous system (essential lesions)
- 2 There are often associated visceral lesions, such as cystic disease of the kidney, the pancreas and the liver

#### XII Angioblastic meningioma

- 1 It grossly resembles the more common types of meningioma
- 2 It is often invasive, manifesting rapid growth
- 3 Morphologically it is similar to other forms of angioblastoma

## XIII Angioglioma?

- 1 A nodule in a cyst wall or a solid tumor
- 2 It contains visible calcium deposits
- 3 It is composed of glial and vascular elements
- 4 There are numerous thick-walled vessels, which are often calcified and are embedded in glial tissue (usually fibrous astrocytes)

## XIV Arterial (racemose) angioma?

- 1 A tangled collection of coiled vessels
- 2 All the vessels must display arterial structure

Since a complete classification based on the different types of anatomic structure of vascular tumors or malformations has never been published, a comprehensive pathologic classification is needed. The simple listing of the various pathologic forms of vascular lesions presented here is adequate if the same term is always applied to the same vascular picture regardless of the clinical features. There are a number of ways to arrange the various types of vascular tumors in forming a classification. They are all more or less acceptable if they are based on the different types of anatomic structure of the lesions. The nosologic grouping which is the most consistent with both the gross and the microscopic nature of these lesions no doubt would furnish the greatest contribution toward an understanding of the pathology of the vascular tumors. The results of the present study suggest that the following classification is more closely correlated with the types of vascular structure (gross and microscopic) than the other possible arrangements.

PATHOLOGIC CLASSIFICATION OF THE  
INTRACRANIAL VASCULAR TUMORS  
AND MALFORMATIONS

## I Angiomatous lesions (comprised of adult vascular elements)

- 1 Parenchymal angioma
  - (a) Telangiectasis (capillary lesion) Rendu-Osler disease
  - (b) Parenchymal varix or varicosity (single or multiple)
  - (c) Cavernous angioma or cavernoma
  - (d) Venous (racemose) angioma
  - (e) Arteriovenous (racemose) angioma
  - (f) ?Arterial (racemose) angioma
- 2 Meningeal angioma
  - (a) Angiomatous meningioma
  - (b) Meningeal varix or varicosity leptomeningeal varix, dural varix, sinus pericranii associated with intracranial angioma(?)
  - (c) Sturge-Weber disease associated with angioma of the meninges

## II Angioblastic lesions (comprised of embryonic vascular elements)

- 1 Parenchymal angioblastoma
  - (a) Lindau's disease (von Hippel-Lindau disease)
- 2 ?Angioglioma

## III Miscellaneous vascular lesions

- 1 Sinus pericranii that is mainly extracranial
- 2 ?Angioglioma
- 3 Sturge-Weber disease without angiomatous lesions

In this classification a majority of the vascular tumors are divided into two main groups according to definite distinctions in their actual anatomic structure. One includes lesions comprised of blood vessels of an adult type and the other includes tumors composed of embryonic vascular elements. The third group is reserved for a few remaining conditions not definitely belonging in the foregoing sections. Then the two main groups are again divided into lesions of the meninges and lesions of the brain parenchyma. Lesions implicating both the meninges and the cerebral tissue are considered as parenchymal lesions since the extent of the neural involvement greatly outweighs the meningeal in significance. These steps are readily apparent except in a few instances, which are indicated by question marks. Sinus pericranii is classified in two different ways since it appears to be mainly extracranial in some cases and intracranial in others. Sturge-Weber disease when associated with angioma of the meninges is placed with the other angiomatous tumors of the meninges while Sturge-Weber disease without such vascular involvement is regarded as a miscellaneous or related condition. The tumors that have been called angiosarcoma are not given a separate heading since they all seem to be classifiable as angioblastic meningioma (see section entitled "Angiosarcoma," page 54).

This classification is submitted with the hope of stimulating a greater consideration of the pathologic structure in further investigations of the intracranial vascular lesions, thereby increasing understanding of the relationship between their clinical and their pathologic aspects. Only if the basic pathologic character is kept in mind will a clearer conception of the correlation between the clinical, the physiologic and the morphologic features of these lesions eventuate. Moreover, there is little doubt that continued inquiries of this kind will increase the ability of physicians to predict the structure of a vascular lesion from its clinical picture.

## Book Reviews

**A Symposium on Mammary Tumors in Mice**  
Publication of the American Association for the Advancement of Science no 22 By Members of the Staff of the National Cancer Institute, National Institute of Health, United States Public Health Service Edited by Forest Ray Moulton Pp 223, illustrated Washington, D C, 25, American Association for the Advancement of Science, 1945

The Symposium consists of twelve papers contributed by ten members of the staff of the National Cancer Institute who have been engaged over several years in the study of tumors of the breast as these develop spontaneously or are induced experimentally in mice belonging to various highly inbred strains. The contributors, who write with the authority derived from a prolonged study of the various problems, have succeeded in their object, which, as stated by M B Shimkin in a "General and Historical Introduction," is "an attempt to collect, to evaluate and to synthesize so far as possible information concerning a specific neoplasm in a specific species of mammal. The major purpose of the symposium is to acquaint the workers in cancer research and those more casually interested in the field with the advances made in this problem.

The first group, consisting of four papers, is concerned with the morphology of the murine mamma and of its epithelial neoplasms. A short paper entitled "The Cytology of Mammary Tumors" by A J Dalton describes mainly the Golgi apparatus. A second paper by the same author entitled "Histogenesis of the Mammary Gland of the Mouse" describes the reaction of the gland to various conditions, such as diet, hormones, pregnancy and lactation, as determined by studying the gland in whole mounts. A third short paper, on the vascular supply of the carcinoma of the mammary gland, by G H Algire and H W Chalkley is essentially an account of a technic for studying the vascular supply of this neoplasm when it develops either spontaneously or after transplantation. These three papers do not quite conform to the plan of the book since, interesting though they are, they are essentially accounts of original studies in limited fields. The fourth paper, however, entitled "Morphology and Histogenesis of Mammary Tumors," by T B Dunn gives a comprehensive account of the subject. Of particular interest is the comparison of the morphologic characters of spontaneous mammary tumors developing in inbred strains with the descriptions by the earlier workers who examined the spontaneous neoplasms arising in the mammae of mice of mixed parentage. The four papers are illustrated by excellent photomicrographs.

The next group, consisting of three papers, relates to the causes of mammary cancer in inbred mice. W E Heston's contribution on the genetics of mammary tumors records in detail the history and the character of the various inbred strains that have been developed, and discusses some of the results obtained from a study of these strains. This systematic treatise in which a worker intimately familiar with the subject has brought together all the relevant data scattered through the literature will be welcomed by all who are engaged in the experimental study of cancer. It is accompanied

by a bibliography comprising nearly two hundred references. The statement on page 56 that "different types of tumors are inherited as separate characters" reads like a lapse of the pen. Are tumors inherited? Does it not mean that the various organs inherit a susceptibility to the development of neoplasms as separate factors? It might have been added here that the recognition of the limitation of an inherited susceptibility to one particular organ enabled Waaler, in Norway, and Wassink, in Holland, to reveal the existence in man of inherited factors in the causation of cancer of the breast.

The complex and fascinating story of the discovery of the so-called milk factor is fully and clearly told in a review of H B Andervont. Although the existence of this factor was recognized only ten years ago, no less than one hundred papers are quoted in the bibliography. The term "milk factor" seems now to have been replaced by the term "milk influence," and so one comes on a section with the curious title "Physical and Chemical Properties of the Milk Influence." From the evidence available at present Andervont argues that the milk influence suggests the action of an agent "belonging or related to the viruses." While indicating the similarities between the milk influence and the viruses, he recognizes the long latent period of the milk influence as a dissimilarity.

The discovery of the milk influence as a decisive factor in the causation of mammary cancer has greatly diminished the significance of the part formerly attributed to the estrogenic hormone. It appears now that this hormone is concerned mainly with building up mammary gland tissue on which the milk influence can act. But there is the apparent paradox that the development of cancers in other organs, e g, the uterine cervix, in response to this hormone is not dependent on the milk influence. Even in the mamma itself cancer can be induced in the absence of the milk influence by the local application of chemical carcinogens such as methylcholanthrene. This complex subject is clearly set out in a paper by M B Shimkin entitled "Hormones and Mammary Cancer in Mice." But the significance of the fact that there are two different etiologic types of mammary cancer in mice should have received more emphasis than is given to it in this account or in the final paper by Shimkin. There arises now the question to which etiologic type the mammary cancer belongs that develops in mice of mixed parentage. This could be determined by testing for the presence or the absence of the milk influence in such mice. The mice of mixed parentage reproduce more closely the conditions responsible for the development of cancer of the breast in women than highly inbred mice. The fact that in inbred strains multiple mammary cancers are much more frequent than in mice of mixed parentage is an indication that such etiologic differences exist.

The degree to which the development of mammary tumors and their subsequent growth can be influenced by such environmental factors as diet, aging and climatic conditions is discussed by H P Morris. Some extravagant statements have been made in the literature concerning the influence of diet, but the author has succeeded in separating the grain from the chaff. In addition to a detailed account of the effect of diet on the development and on the growth of cancer this sec-

tion provides a variety of interesting and valuable data which hitherto were scattered through the literature. An example is the frequency with which synchronous multiple mammary carcinoma is found in mice of mixed parentage and in mice belonging to inbred strains. Thus, in the early work of Haaland in mice of mixed parentage 17 per cent of the cancerous mice had multiple carcinoma, while in the more recent observations on two different inbred strains multiple carcinoma was found in 66 and 61.5 per cent of all cancerous mice.

The relation between age and the incidence of mammary cancer in inbred strains receives detailed treatment in which a clear distinction is made between the crude data (the number of deaths from cancer at different ages) and the death rates calculated from these data. The irregularities appearing in the older age groups are due to the small number of animals alive in those groups and also to the shortness of the age periods, single months, into which the normal span of life of the mouse has been divided in this paper. In cancer statistics applying to man age periods of ten years are used as a rule. These correspond to periods of three months in the shorter life span of the mouse. If the mortality rates per thousand were recalculated for three monthly periods the results obtained would resemble even more closely the conditions existing in man, namely, an increase which is progressively rapid.

In the chapter on the chemistry of mammary tumors, I. P. Greenstein has succeeded in condensing a vast amount of information into six pages of text, an achievement that will be welcomed by nonchemists. In addition to the chemistry of the tumors, information is given on the chemistry of the tissues of tumor-bearing animals and on different strains of mice. The surprising fact emerges that the degree of activity of one ferment, the serum esterase, which shows considerable differences between various strains, can be altered by foster nursing, so that the fostered mice have an activity resembling that of the strains to which the foster mother belongs. Greenstein summarizes the results of these investigations in the generalization that the end results of all forms of neoplastic growth are tissues which are chemically similar. Enzymatically tumors resemble one another more than they resemble normal tissues and more than normal tissues resemble one another.

The experimental work on the therapy of cancer, so far as it has been studied on mammary cancer of mice, is critically reviewed by H. M. Ryer. It is surprising to find that no less than 800 substances of various kinds have been tested for their therapeutic effect. Most of these have yielded completely negative results, but in spite of this melancholy fact the paper, which must have entailed a laborious search through the literature, makes interesting reading. The data are summarized in a tabular form most useful for purposes of reference.

The Symposium ends with a paper by M. B. Shimkin entitled "Conclusions—Including Discussion of the Possible Implications for Man." It sums up clearly and succinctly the results that have been obtained from a study of cancer of the breast in inbred strains of mice. But when the writer passes on to a discussion of their implications he goes sadly astray. He states (page 220) "It is plain that the designations of 'benign,' 'precancerous' or 'malignant' are clinical terms necessary for pragmatic purposes of selecting a therapeutic procedure and establishing prognosis but not applicable to experimental work."

I cannot find either in the Symposium or in the experimental work on cancer as this is known to me anything that would justify this extravagant, one might

almost call it reckless, statement. If it refers to the inability to demonstrate a clearcut morphologic difference between cancerous cells and noncancerous cells or between precancerous and nonprecancerous conditions, it refers to difficulties long recognized by pathologists. B. Bloch, who was both a clinician and a pioneer in the experimental study of carcinogenesis and whose work helped to establish the conception of precancer, emphasized the fact that there may be no morphologic distinction between a precancerous hyperplasia and a nonprecancerous hyperplasia. Cancer cells betray their presence by their biologic activity, namely, their power of autonomous invasive growth. What the experimental work in cancer has accomplished is the demonstration that this power is possessed only by cancer cells and not by cells present in hyperplastic or precancerous tissues. I find it difficult to believe that Dr. Shimkin wants pathologists to discard the conception of autonomous invasive growth as representing the essential nature of cancer. Nor should pathologists ignore the vast amount of work on human cancer that has made it possible to establish correlations between the morphologic appearances and the biologic behavior of a tumor. Whatever the meaning of the sentence quoted may be, it is unfortunately phrased.

On page 214, attention is drawn to the fallacy of assuming that statistical correlation implies causal or other direct connection. The fallacy is cleverly illustrated by the wearing of skirts, which "would undoubtedly correlate strongly with the occurrence of mammary cancer, yet has no possible direct significance in the etiology of such tumors." On the very next page, however, reference is made to the statistical correlation between the increase in age and the increase of incidence of mammary cancer, and it is argued, "Age in itself therefore is a predisposing factor in mammary cancer in man." This view was generally held until the discovery of experimental carcinogenesis made it possible to test its validity. When young and old mice were subjected to the experimental test, the alleged greater susceptibility failed to manifest itself. The susceptibility was either the same for both or higher in the young animals.

As a general proposition the explanation of the higher incidence of cancer with increasing age must therefore be abandoned. How, then, can this feature of cancer be explained? One factor which contributes to this phenomenon is the long period of induction of cancer, which becomes increasingly prolonged as the strength of the carcinogenic stimulus is diminished. This point is of more than academic interest. One cannot escape from old age, but it is possible to prolong the period of induction. That makes it possible to push the onset of cancer more and more into the older age groups. Such a result, which may be described as a halfway house to the prevention of cancer, would constitute a great advance in the control of the disease. But its possibility is not likely to be recognized so long as old age is in itself considered to be an etiologic factor.

Since in the experimental investigation of cancer the mamma of the mouse has been used exclusively as a test object, this book with its collection and ordered presentation of so many relevant data is likely to exert considerable influence on oncologic thought. It is a "must" for every cancer worker and a "should" for every pathologist and clinician who wants to keep abreast of the rapid advances in knowledge of the biology of cancer. This detailed, if in parts critical, review is a sincere tribute to its importance.

# INDEX TO VOLUME 39

Subject entries are made for all articles. Author entries are made for original articles. Book Reviews and Obituaries are indexed under these headings in alphabetical order under the letters B and O, respectively.

**Abdomen** See Gastrointestinal Tract, etc  
**Abnormalities and Deformities** See Monsters, and under names of organs and regions, as Aorta, Cranium, etc  
**Abortion**, effects of abortifacient paste ("ultra-jel"), report of death from its use and of experimental study of its effects on rabbits and rats 91  
**Abscess** See under names of organs and regions  
**Acacia atheromatosis**, 121  
**Acid Amino** See Amino Acids  
**Adenocarcinoma**, changes in uterus after eradication of endometrial adenocarcinoma by radiotherapy with reference to infarct-like radionecrotic plaque in lining 237  
**Adenoma**, adrenal cortical adenoma of epididymis, 336  
     of hypophysis 343  
**Adenomyoepithelioma (cylindroma) of palatal mucous glands**, 96  
**Adipose Tissue** See Fat  
**Adrenals** adrenal cortical adenoma of epididymis 336  
     morbidity anatomy of typhus as seen in recent Guatemalan epidemic observations on disease, 226  
**Agranulocytosis** acute, microscopic study of ulceronecrotic gingiva and of bone marrow in case, 369  
**Alcohol**, polyvinyl alcohol atheromatosis, 118  
**Amino Acids**, morphologic studies of rats deprived of essential amino acids, phenylalanine 294  
**Amyloidosis**, 207  
     primary systemic 315  
**Anaphylaxis and Allergy** See also Asthma, etc  
     inflammatory vascular reactions, allergy 209  
     pathology of anaphylaxis due to sulfonamide drugs 301  
**Anday, G. J.** Acute agranulocytosis, microscopic study of ulceronecrotic gingiva and of bone marrow in case, 369  
**Aneurysm** periarteritis nodosa producing aneurysm of renal artery and hypertension, 274  
**Angina** Agranulocytic See Agranulocytosis  
**Angioblastoma cerebellar** 408  
**Angioglioma**, 414  
**Angioma** angiomatous meningioma, 406  
     arterial (racemosum) 404  
     arteriovenous 404  
     cavernous, 401  
     Lindau's (von Hippel's) disease 411  
     Sturge-Weber disease 407  
     venous 403  
**Angiosarcoma**, 413  
**Anoxemia** See Blood, oxygen  
**Antibodies** See Antigens and Antibodies  
**Antigens and Antibodies** See also Lipoids, and under specific antigens and reactions  
     studies on pathogenesis of glomerulonephritis, production of autoantibodies to kidney in experimental animals 148  
**Antiserum** See under names of various diseases  
**Aorta** abnormalities in dogs used for experimental purposes, 375  
     Aneurysm See under Aneurysm  
     coarctation with local hydrostatic hypertension, 56  
     ossified cartilage with myeloid fat marrow in aortic ring of rabbit 89  
**Appointments**, 66 131 280 350  
**Arteries** See also Aneurysm, Aorta, Arteriosclerosis, Blood pressure, Periarteritis, etc  
     Hypertension See Blood pressure, high  
     inflammation, temporal arteritis 211

## Arteries—Continued

Renal See under Kidneys  
     theory of transposition of arterial trunks based on phylogenetic and ontogenetic development of heart 172  
**Arteriosclerosis**, 51, 117, 187  
     acacia atheromatosis, 121  
     aortic abnormalities in dogs used for experimental purposes, 375  
     attempts to produce cerebral atherosclerosis 16  
     colloidal plasmatic disturbances, carbohydrate type, 117  
     colloidal plasmatic disturbances, lipoidal type, 122  
     coronary, and myocardial infarction as studied by injection technic, 268  
     correlation between chemical and morphologic alterations in experimental atherosclerosis, 11  
     excessive intake of cholesterol, 187  
     glycogenic vascular reactions, 121  
     methyl cellulose atheromatosis, 120  
     pectin atheromatosis, 121  
     polyvinyl alcohol atheromatosis, 118  
     preventive and therapeutic aspects 211  
     sclerosis of pulmonary artery and its branches 58  
**Arteritis** See Arteries, inflammation  
**Ashburn, L. L.** Experimental Haplosporangium infection, 3  
     Tularemia report of laboratory infection fatal on fifth day with early pulmonary involvement autopsy, 388  
**Asthma** See also Anaphylaxis and Allergy  
     bronchial and tuberculosis 338  
**Atheroma** See Arteriosclerosis  
**Atheromatosis** See Arteriosclerosis  
**Atherosclerosis** See Arteriosclerosis  
**Atrophy** See under names of organs and regions  
**Awards**, 66, 350  
**Bacteria** See under Streptococci, etc  
     Tularense See Tularemia  
**Batchelor, T. M.** Congenital glycogenic tumors of heart, 67  
**Bauer, W. H.** Adenomyoepithelioma (cylindroma) of palatal mucous glands, 96  
**Baumann, E. J.** Periarteritis nodosa-like lesions in rats fed thioracil, 325  
**Bile Ducts** cholesterol and biliary obstruction, 127  
**Bilharziasis** See Schistosomiasis  
**Biliary Tract** See Bile Ducts, Liver  
**Black-Schaffer, B.** Pathology of anaphylaxis due to sulfonamide drugs, 301  
**Blood** See also Hemoglobin and Hemoglobin Compounds  
     colloidal plasmatic disturbances, proteinic type 205  
     correlation between chemical and morphologic alterations in experimental atherosclerosis 11  
     Diseases See Anemia, etc  
     oxygen anoxemia theory 51, 117, 187  
     oxygen hematic anoxemia, 213  
     pressure high, experimental hypertension its production in dogs by intravenous injection of streptococci 81  
     pressure, high, general hydrostatic hypertension or plethora, 55  
     pressure high, local hydrostatic hypertension in arteriovenous fistula 55  
     pressure high, local hydrostatic hypertension, physical labor and static and gravitational factors 56  
     pressure high, local hydrostatic hypertension with coarctation of aorta 56  
     pressure high, periarteritis nodosa producing aneurysm of renal artery and hypertension 274  
**Vessels** See Arteries Periarteritis, etc

- Bones See also under names of bones  
combined effects of estrogen and anterior hypophyseal extract on skeleton of growing mouse, 381  
marrow, acute agranulocytosis, microscopic study of ulceronecrotic gingiva and of bone marrow in case 369  
marrow, ossified cartilage with myeloid fat marrow in aortic ring of rabbit, 89
- BOOK REVIEWS  
Atlas of Blood in Children, A. D. Blackfan and L. K. Diamond 66  
Avitaminoses Chemical Clinical and Pathological Aspects of Vitamin Deficiency Diseases, W. H. Eddy and G. Dalldorf 219  
Biological Basis of Individuality I. Loeb, 217  
Bronchial Asthma, L. Unger, 350  
Lead Poisoning A. Cantarow and M. Frumper 220  
Pathology of Internal Diseases W. Boyd 219  
Spina Bifida and Cranium Bifidum Papers Reprinted from New England Journal of Medicine with Addition of Comprehensive Bibliography, from Department of Surgery of Children's Hospital, Boston and Harvard Medical School F. D. Ingraham, 220  
Symposium on Mammary Tumors in Mice edited by F. R. Moulton, 417  
Textbook of Pathology, R. A. Moore 218  
Trauma in Internal Diseases with Consideration of Experimental Pathology and Medical Aspects, R. A. Stern, 350
- Books received 220, 280 392
- Brain See also Cerebellum Meninges Nervous System etc  
attempts to produce cerebral atherosclerosis 16  
cerebral varix, 399  
diffuse meningeal fibroblastoma of brain and spinal cord, report of 3 cases, 287  
paraphyseal cyst of third ventricle 184
- Brandes W. W. Presacral cyst apparently arising from neurenteric canal in newborn infant 267
- Breast mammary neoplasms in dogs 169
- Bright's Disease See Nephritis
- Bronchi cysts 155  
two cases of congenital web of bronchus 47
- Cahill, W. M. Morphologic studies of rats deprived of essential amino acids, phenylalanine 294
- Cancer See Adenocarcinoma, Sarcoma Tumors etc and under names of organs and regions, as Esophagus, Liver, Thyroid etc
- Canines See Dogs
- Carbohydrates See also Glycogen, etc  
colloidal plasmatic disturbances carbohydrate type 117
- Carbon Disulfide and hydrogen sulfide poisoning 130
- Cardiovascular System See Arteries Heart etc
- Ceres R. Medialastinal teratoma, 113
- Cartilage, ossified, with myeloid fat marrow in aortic ring of rabbit, 89
- Cattle polkilocytosis in dairy cattle 351
- Cavelti E. S. Studies on pathogenesis of glomerulonephritis, production of autoantibodies to kidney in experimental animals 148
- Cavelti P. A. Studies on pathogenesis of glomerulonephritis, production of autoantibodies to kidney in experimental animals, 148
- Cells adenomyoepithelioma (cylindroma) of palatal mucous glands 96  
proliferation of muscle cells in myometrium of non-pregnant uterus, 323
- Cellulose, methyl cellulose atheromatosis, 120
- Cerebellum angioloblastoma, 408
- Cervix See Uterus
- Chemotherapy See under names of diseases and chemotherapeutic agents
- China and Chinese role of schistosomiasis in etiology of cancer of liver in Chinese, 1
- Cholesterol and biliary obstruction, 127  
attempts to produce cerebral atherosclerosis 16  
carbon disulfide and hydrogen sulfide poisoning, 130  
chronic renal disease and hypercholesterolemia, 130  
colloidal plasmatic disturbances, lipoidal type, 122  
correlation between chemical and morphologic alterations in experimental atherosclerosis 11
- Cholesterol—Continued  
excessive intake of 187  
hypercholesterolemia and diabetes mellitus 128  
hypercholesterolemia and tuberculous xanthomatosis 127  
hypothyroidism, 128  
physical labor and hypercholesterolemia, 131  
saponin poisoning and hypercholesterolemia 131
- Christian-Weber Disease See Panniculitis
- Chromosomes genetic analysis of induction of tumors by methylcholanthrene 2 mutations arising in mice following injection of methylcholanthrene 232
- Coccidioidomycosis, experimental Haplosporangium infection 3
- Colon See Gastrointestinal Tract, Intestines
- Corpora Amylacea See Amyloidosis
- Cranio-pharyngioma of hypophysis 346
- Cranium, intracranial vascular tumors and malformations, 393  
sinus pericranii 401
- Cylindroma, adenomyoepithelioma (cylindroma) of palatal mucous glands 96
- Cysts See under names of organs and regions as Brain, Bronchi, Esophagus, Intestines Mediastinum, Stomach, etc  
Dermoid See under Tumors
- Cytology See Cells
- Davis R. M. Morphologic studies of rats deprived of essential amino acids, phenylalanine 294
- Death effects of abortifacient paste ('ultra-jel') report of death from its use and of experimental study of its effects on rabbits and rats 91
- Degeneration, Amyloid See Amyloidosis
- De Nosaquo N. Effects of abortifacient paste ('ultra-jel'), report of death from its use and of experimental study of its effects on rabbits and rats 91
- Diabetes Mellitus and hypercholesterolemia, 128
- Dick, G. F. Experimental hypertension its production in dogs by intravenous injection of streptococci, 81
- Dogs aortic abnormalities in dogs used for experimental purposes 375  
some endocrinologic considerations of canine neoplastic diseases 162
- Ductless Glands See Endocrine Glands
- Duncan C. W. Polkilocytosis in dairy cattle 351
- Dunn, R. C. New hemoglobin stain for histologic use slightly modified Van Gieson stain 49
- Eckhardt, R. L. Myelomalacia of cervical portion of spinal cord, probably result of roentgen therapy 109
- Edema See under names of organs and regions
- Electricity pancreatic necrosis in electric shock 9
- Emmons C. W. Experimental Haplosporangium infection 3  
Phialophora jeanselmii comb. n. from mycetoma of hand 364
- Endocardium See Heart
- Endocrine Glands neoplasms of other endocrine glands 167  
some endocrinologic considerations of canine neoplastic diseases 162
- Endometrium, changes in uterus after eradication of endometrial adenocarcinoma by radiotherapy with reference to infarct-like radionecrotic plaque in lining 237
- Enteritis See under Intestines
- Epidermis See Skin
- Epididymis adrenal cortical adenoma of 336
- Erythrocytes See Anemia, Blood Hemoglobin and Hemoglobin Compounds etc
- Esophagus, carcinoma in British West Indian and Panamanian Negroes, study of incidence etiological factors and pathologic anatomy in 50 cases, 79  
cysts 158
- Estrodiol Benzoate See Estrogens
- Estrogens combined effects of estrogen and anterior hypophyseal extract on skeleton of growing mouse, 381
- Evolution theory of transposition of arterial trunks based on phylogenetic and ontogenetic development of heart, 172



- Fat** See also Lipoids  
ossified cartilage with myeloid fat marrow in aortic ring of rabbit, 89
- Felix-Well Reaction** See Typhus
- Fellowships**, 350
- Fernan-Nunez, Marcos** resignation from Marquette University, 280
- Fever** See also Typhus, etc  
chemical basis of fever with inflammation, 28  
therapy See under names of diseases
- Fibroblastoma** diffuse meningeal fibroblastoma of brain and spinal cord, report of 3 cases, 287
- Filum Terminale** See Spinal Cord
- Fistula**, arteriovenous, local hydrostatic hypertension in 55
- Fite, G L** Classification of tumors of kidney 37
- Fox, R A** Adenomyoepithelioma (cylindroma) of palatal mucous glands, 96
- Freeman A** Adrenal cortical adenoma of epididymis 336
- Friedman, N B** Fatal panniculitis, 42
- Fungi**, experimental Haplosporangium infection, 3  
experimental reproduction of maduromycotic lesions in rabbits, 358  
Phialophora jeanselmei comb n from mycetoma of hand, 364
- Ganglion** See also Nervous System, Neurons  
nodosum, histologic diagnosis of rabies, 279
- Gastrointestinal Tract** See also Intestines, Stomach, etc  
gastroenteric cysts, 158
- Genetics** See Chromosomes
- Genitals** See also under names of genitals  
neoplasms of female genital tract 167  
neoplasms of male genital tract, 165
- Gingivitis** See Gums
- Glazer A M** Pancreatic necrosis in electric shock 9
- Glioma** morphologic alterations of neuron due to tumor invasion, 221
- Glomerulonephritis** See Nephritis
- Glycogen**, congenital glycogenic tumors of heart 67  
glycogenic vascular reactions 121
- Golden A** Morbid anatomy of typhus as seen in recent Guatemalan epidemic, observations on disease, 226
- Gotwals J E** Ischiopagus tripus, report of 2 cases 142
- Grants**, 131
- Granulocytopenia** See Agranulocytosis
- Granuloma Coccidioidale** See Coccidioidomycosis  
experimental Haplosporangium infection, 3
- Gums** acute agranulocytosis, microscopic study of ulceronecrotic gingiva and of bone marrow in case, 369
- Gurley, H** Experimental nephropathies, renal phosphatase after poisoning with mercury bichloride, uranyl nitrate and potassium dichromate, 133
- Hand, Phialophora jeanselmei** comb n from mycetoma of, 364
- Haplosporangium** See Fungi
- Hartz, P H** Proliferation of muscle cells in myometrium of nonpregnant uterus, 323  
Role of schistosomiasis in etiology of cancer of liver in Chinese, 1
- Hay Fever** See Anaphylaxis and Allergy
- Haythorn, S R** Diffuse meningeal fibroblastoma of brain and spinal cord, report of 3 cases, 287
- Heart** See also Pericarditis, Pericardium  
congenital glycogenic tumors of, 67  
coronary arteriosclerosis and myocardial infarction as studied by injection technic 268  
primary systemic amyloidosis, 315  
primary tumor of, 74  
theory of transposition of arterial trunks based on phylogenetic and ontogenetic development of, 172
- Hemoglobin and Hemoglobin Compounds** See also Anemia Blood  
new hemoglobin stain for histologic use, slightly modified Van Gieson stain, 49
- Hepatic Duct** See Bile Ducts
- Hepatitis** See Liver
- Hepler, O E** Experimental nephropathies, method of producing controlled selective injury of renal units by means of chemical agents 103
- Hepler, O E**—Continued  
Experimental nephropathies, renal phosphatase after poisoning with mercury bichloride, uranyl nitrate and potassium dichromate, 133
- Herbut P A** Bronchial asthma and tuberculosis 338  
Melanoma of small intestine 22  
Periarteritis nodosa producing aneurysm of renal artery and hypertension, 274
- Heredity**, genetic analysis of induction of tumors by methylcholanthrene, 2 mutations arising in mice following injection of methylcholanthrene, 232
- Herzog E** Histologic diagnosis of rabies, 279
- von Hippels Disease** See Angioma, Retina
- Hirsch E F** Chronic exudative and indurative pneumonia due to inhalation of shellac, 281
- Holyoke J B** Coronary arteriosclerosis and myocardial infarction as studied by injection technic 268
- Hormones** See Endocrine Glands, Estrogens, Pituitary Preparations etc
- Hueper, W C** Aortic abnormalities in dogs used for experimental purposes, 375  
Arteriosclerosis, 51, 117 187  
Ossified cartilage with myeloid fat marrow in aortic ring of rabbit, 89
- Huffman C F** Polkilocytosis in dairy cattle, 351
- Hyalinosis**, noninflammatory vascular reactions, 206
- Hydrogen Sulfide** carbon disulfide and hydrogen sulfide poisoning 130
- Hydrophobia** See Rabies
- Hypercholesteremia** See under Cholesterol
- Hyperergy** See Anaphylaxis and Allergy
- Hyperpyrexia** See Fever
- Hypertension** See Blood pressure, high
- Hyperthermia** See Fever
- Hypertonia** See Blood pressure high
- Hypophysis** See Pituitary Body
- Hypothyroidism** See under Thyroid
- Ileum** See under Intestines
- Immunity** See Anaphylaxis and Allergy Antigens and Antibodies, etc
- Indexes**, importance of titles that accurately reflect subjects of articles, 132
- Industrial Diseases** chronic exudative and indurative pneumonia due to inhalation of shellac, 281
- Infants, Newborn** See Newborn Infants
- Infarction** See Heart
- Infection** See under names of bacteria as Streptococci etc
- Inflammation** chemical basis of fever with inflammation 28
- Injections**, coronary arteriosclerosis and myocardial infarction as studied by injection technic 268
- Internal Secretions** See Endocrine Glands
- Intestines** See also Gastrointestinal Tract  
enteric cysts 159  
melanoma of small intestine, 22
- Intranuclear Inclusions** See under Cells
- Irradiation** See under names of diseases and organs
- Ischiopagus Tripus** See Monsters
- Islands of Langerhans** See Pancreas
- Jejunum** See Intestines
- Kidneys, Diseases** See also Nephritis  
diseases, chronic renal disease and hypercholesteremia, 130  
experimental hypertension, its production in dogs by intravenous injection of streptococci, 81  
experimental nephropathies, method of producing controlled selective injury of renal units by means of chemical agents, 103  
experimental nephropathies, renal phosphatase after poisoning with mercury bichloride, uranyl nitrate and potassium dichromate 133  
periarteritis nodosa producing aneurysm of renal artery and hypertension 274  
studies on pathogenesis of glomerulonephritis production of autoantibodies to kidney in experimental animals 148  
tumors classification of, 37
- Knorr, W F** Primary systemic amyloidosis 315
- Koessler Jessie Horton** Fellowship 350
- Kraus J E** Neoplastic diseases of human hypophysis, 343
- Kussmaul-Maier Disease** See Periarteritis nodosa

- Laipply, T C** Cysts and cystic tumors of mediastinum 153
- Langerhans Islands** See Pancreas
- Lav, M** Theory of transposition of arterial trunks based on phylogenetic and ontogenetic development of heart, 172
- Lindau's Disease** See Angioma, Retina
- Lindsay, S** Primary systemic amyloidosis, 315
- Lipids** See Fat, Lipoids
- Lipoids** See also Cholesterol Fat, etc  
colloidal plasmatic disturbances, lipoidal type, 122
- Literature**, importance of titles that accurately reflect subjects of articles, 132
- Liver** cancer role of schistosomiasis in etiology in Chinese 1
- Lungs** experimental *Haplosporangium* infection 3  
latent primary carcinoma of thyroid gland, 331  
tularemia, report of laboratory infection fatal on fifth day with early pulmonary involvement, autopsy, 388
- Lymphangioma**, cystic, 159
- Madura Foot** See Mycetoma
- Maduromycosis** See Mycetoma
- Mayer-Rusmann Disease** See Periarthritis nodosa
- Malformations** See Monsters, and under names of organs and regions
- Mammary Gland** See Breast
- Manges, W E** Melanoma of small intestine 22
- Marine, D** Periarthritis nodosa-like lesions in rats fed thiouracil 325
- Martland, H S** Importance of titles that accurately reflect subjects of articles 132
- Maun, M E** Congenital glycogenic tumors of heart 67  
Morphologic studies of rats deprived of essential amino acids phenylalanine 291
- Mediastinum**, cysts and cystic tumors of, 153  
teratoma 113
- Meinicke Reaction** See under Tuberculosis
- Melanoma** of small intestine, 22
- Meninges** diffuse meningeal fibroblastoma of brain and spinal cord, report of 3 cases, 287  
vascular tumors of 396
- Meningioma** angloblastic, 412  
angiomatous, 406
- Minkin, V** Chemical basis of fever with inflammation 28
- Mercury**, experimental nephropathies renal phosphatase after poisoning with mercury bichloride, uranyl nitrate and potassium dichromate 133
- Merliss, R** Primary tumor of heart, 71
- Metals** See also Mercury, etc  
experimental nephropathies, method of producing controlled selective injury of renal units by means of chemical agents, 103
- Methyl Cellulose** See Cellulose
- Methylcholanthrene**, healing of wounds in skin of mice painted with 20-methylcholanthrene 257  
genetic analysis of induction of tumors by 2 mutations arising in mice following injection of methylcholanthrene 232
- Miller, S E** Tularemia, report of laboratory infection fatal on fifth day with early pulmonary involvement, autopsy 388
- Mitchell, N** Latent primary carcinoma of thyroid gland 331
- Molds** See Fungi
- Monsters** *ischlopagus tripus* report of 2 cases, 142
- Moss, L D** Paraphysal cyst of third ventricle, 184
- Mucous Membrane** See under Palate, etc
- Mulligan, R M** Some endocrinologic considerations of canine neoplastic diseases, 162
- Muscles**, proliferation of muscle cells in myometrium of nonpregnant uterus, 323
- Mutation** See Heredity
- Mycetoma** experimental reproduction of maduro-mycotic lesions in rabbits 358  
*Phialophora jeanselmei* combination from mycetoma of hand, 364
- Myeloma**, myelomatosis 206
- Myelomalacia** See Spinal Cord
- Myocardium** See Heart
- Myoma**, proliferation of muscle cells in myometrium of nonpregnant uterus 323
- Myometrium** See Uterus
- Necrosis** See under Pancreas
- Negri Bodies** See Rabies
- Negros**, esophageal carcinoma in British West Indian and Panamanian Negros, study of incidence etiological factors and pathologic anatomy in 50 cases, 79
- Necroplasms** See Sarcoma Tumors
- Nephritis** See also Kidneys, diseases  
studies on pathogenesis of glomerulonephritis, production of autoantibodies to kidney in experimental animals, 118
- Nephropathy** See under Kidneys
- Nephrosis** See Kidneys diseases Nephritis
- Nerves** See Nervous System
- Cells** See Neurons
- Nervous System** See also Brain, Cerebellum, Spinal Cord, etc  
morbid anatomy of typhus as seen in recent Guatemalan epidemic, observations on disease, 226  
morphologic alterations of neuron due to tumor invasion 221
- Neurenteric Canal**, presacral cyst apparently arising from neurenteric canal in newborn infant, 265
- Neurohypophysis** See Pituitary Body
- Neurons**, morphologic alterations of neuron due to tumor invasion, 221
- Neutropenia** See Agranulocytosis
- Newborn Infants**, presacral cyst apparently arising from neurenteric canal in 265
- Nomenclature** classification of tumors of kidney 37
- Noran, H H** Intracranial vascular tumors and malformations 393
- Obituaries**  
Arkwright Joseph 171  
Carril Alexis, 66  
Crill, John C, 280  
Edlingham John 66  
Sachs Hans, 350
- Occupational Diseases** See Industrial Diseases
- Odom, G L** Morphologic alterations of neuron due to tumor invasion 221
- Ontogeny** See Evolution
- Orban, B** Acute agranulocytosis microscopic study of ulceronecrotic gingiva and of bone marrow in case 369
- Organs** morphologic studies of rats deprived of essential amino acids phenylalanine, 291
- Ossler's Disease** See Telangiectasia
- Ossification** See under Cartilage
- Palate** adenomyoepithelioma (cylindroma) of palatal mucous glands 96
- Pancreas** necrosis in electric shock 9
- Panniculitis** fatal 42
- Parkes Weber-Christian Disease** See Panniculitis
- Pastes** effects of abortifacient paste (Utra-Jel) report of death from its use and of experimental study of its effects on rabbits and rats, 91
- Pectin** atheromatosis 120
- Periarthritis nodosa** 211  
nodosa-like lesions in rats fed thiouracil 325  
nodosa producing aneurysm of renal artery and hypertension 271
- Pericardium**, pericardial celomic cysts 155
- Permanent Foundation Hospital Fellowship** 50
- Phenylalanine**, morphologic studies of rats deprived of essential amino acids, 291
- Phialophora** See Fungi
- Phosphatase**, experimental nephropathies renal phosphatase after poisoning with mercury bichloride uranyl nitrate and potassium dichromate 133
- Phylogeny** See Evolution
- Pituitary Body** metastatic tumors, 348  
neoplastic diseases of human hypophysis 313
- Pituitary Preparations**, combined effects of estrogen and anterior hypophyseal extract on skeleton of growing mouse, 381
- Plotz, Ella Sachs Plotz Foundation** 131
- Pneumonia** chronic exudative and indurative pneumonia due to inhalation of shellac 281
- Polkilocytosis** in dairy cattle 351
- Poisons and Poisoning** See also under names of specific substances, as Mercury, Saponin, etc  
experimental nephropathies, method of producing controlled selective injury of renal units by means of chemical agents, 103

- Pollak, O J Attempts to produce cerebral atherosclerosis, 16  
Correlation between chemical and morphologic alterations in experimental atherosclerosis, 11  
Pollen See Anaphylaxis and Allergy, etc  
Potassium dichromate, experimental nephropathies, renal phosphatase after poisoning with mercury bichloride, uranyl nitrate and potassium dichromate, 133  
Price, A H Periarthritis nodosa producing aneurysm of renal artery and hypertension, 274  
Prizes See Awards  
Rabies histologic diagnosis of, 279  
Radiations See also Roentgen Rays, and under names of various diseases  
changes in uterus after eradication of endometrial adenocarcinoma by radiotherapy with reference to infarct-like radionecrotic plaque in lining, 237  
Radiotherapy See Radiations  
Reid J T Poikilocytosis in dairy cattle, 351  
Rendu-Osler Disease See Telangiectasia  
Reproduction, grants for work on problems of 131  
Reticuloendothelial System See Anemia, Liver, etc  
Retina Lindau's (von Hippel's) disease, 411  
Roentgen Rays See also Radiations  
myelomalacia of cervical portion of spinal cord probably result of roentgen therapy, 109  
Roentgenotherapy See under names of diseases  
Russell, H B Chronic exudative and indurative pneumonia due to inhalation of shellac, 281  
Sacrococcygeal Region, presacral cyst apparently arising from neurenteric canal in newborn infant, 265  
Saphir O Theory of transposition of arterial trunks based on phylogenetic and ontogenetic development of heart, 172  
Saponin poisoning and hypercholesteremia 131  
Sarcoma See also Angiosarcoma, Tumors, etc  
infectious or venereal, 168  
Schistosomiasis, role in etiology of cancer of liver in Chinese, 1  
Schlumberger, H G Ischiopagus tripus, report of 2 cases, 142  
Schmitz H E Changes in uterus after eradication of endometrial adenocarcinoma by radiotherapy with reference to infarct-like radionecrotic plaque in lining, 237  
Sclerosis See Arteriosclerosis  
Secretions Internal See Endocrine Glands  
Serum See Blood  
Sickness See Anaphylaxis and Allergy  
Sex grants for work on problems of 131  
Shaper, W Diffuse meningeal fibroblastoma of brain and spinal cord, report of 3 cases, 287  
Sheehan, J F Changes in uterus after eradication of endometrial adenocarcinoma by radiotherapy with reference to infarct-like radionecrotic plaque in lining, 237  
Shellac, chronic exudative and indurative pneumonia due to inhalation of, 281  
Shock Anaphylactic See Anaphylaxis and Allergy  
Electric See Electricity  
Silberberg, M Combined effects of estrogen and anterior hypophyseal extract on skeleton of growing mouse, 381  
Healing of wounds in skin of mice painted with 20-methylcholanthrene, 257  
Silberberg, R Combined effects of estrogen and anterior hypophyseal extract on skeleton of growing mouse, 381  
Healing of wounds in skin of mice painted with 20-methylcholanthrene, 257  
Simonds, I P Experimental nephropathies, method of producing controlled selective injury of renal units by means of chemical agents 103  
Experimental nephropathies, renal phosphatase after poisoning with mercury bichloride, uranyl nitrate and potassium dichromate, 133  
Sinus Pericranii See Cranium  
Skeleton See under Bones  
Skin, healing of wounds in skin of mice painted with 20-methylcholanthrene, 257  
Societies, American Association for Cancer Research 131  
Federation of American Societies for Experimental Biology 131  
Societies—Continued  
Public Health Cancer Association, 66  
Society of American Bacteriologists, 131  
Specimens new hemoglobin stain for histologic use, slightly modified Van Gieson stain 49  
Spinal Cord See also Meninges, Nervous System, etc  
diffuse meningeal fibroblastoma of brain and spinal cord, report of 3 cases, 287  
myelomalacia of cervical portion of spinal cord, probably result of roentgen therapy, 109  
Stains and Staining, new hemoglobin stain for histologic use, slightly modified Van Gieson stain, 49  
Storn, K Morphologic alterations of neuron due to tumor invasion, 221  
Stevenson, L D Myelomalacia of cervical portion of spinal cord, probably result of roentgen therapy, 109  
Stewart, H C Diffuse meningeal fibroblastoma of brain and spinal cord, report of 3 cases, 287  
Stomach See also Gastrointestinal Tract  
gastric cysts 158  
Straus, R Effects of abortifacient paste ("utrojel"), report of death from its use and of experimental study of its effects on rabbits and rats, 91  
Primary tumor of heart, 74  
Streptococci, experimental hypertension, its production in dogs by intravenous injection of streptococci, 81  
studies on pathogenesis of glomerulonephritis, production of autoantibodies to kidney in experimental animals, 148  
Strong, L C Genetic analysis of induction of tumors by methylcholanthrene, 2 mutations arising in mice following injection of methylcholanthrene, 232  
Sturge-Weber Disease See Angioma  
Sulfonamides pathology of anaphylaxis due to sulfonamide drugs 301  
Suprarenals See Adrenals  
Sutton J B Presacral cyst apparently arising from neurenteric canal in newborn infant, 265  
Symmers D Experimental reproduction of maduro-mycotic lesions in rabbits 358  
Syphilis See under names of organs, regions and diseases  
Telangiectasia, 397  
Teratoma epidermoid cyst and dermoid cyst, 153  
mediastinal, 113  
Thesauriosis See Cellulose  
Thiouracil, periarthritis nodosa-like lesions in rats fed thiouracil, 325  
Thompson, E C New hemoglobin stain for histologic use, slightly modified Van Gieson stain 49  
Thorax See Heart, Lungs, Mediastinum, etc  
Thyroid, hypothyroidism 128  
latent primary carcinoma of thyroid gland 331  
Tissue See Cells  
Adipose See Fat  
Titles importance of titles that accurately reflect subjects of articles, 132  
Tomlinson W J Esophageal carcinoma in British West Indian and Panamanian Negroes, study of incidence etiologic factors and pathologic anatomy in 50 cases, 79  
Towne, J Changes in uterus after eradication of endometrial adenocarcinoma by radiotherapy with reference to infarct-like radionecrotic plaque in lining, 237  
Tuberculosis and bronchial asthma 338  
Fularemia, report of laboratory infection fatal on fifth day with early pulmonary involvement, autopsy, 388  
Tumors See also Adenocarcinoma, Adenoma, Angioma, Glioma, Lymphangioma, Melanoma, Sarcoma, Teratoma, and under names of organs and regions as Breast, Cranium, Heart, Kidneys, Meninges, Pituitary Body, etc  
classification, intracranial vascular tumors and malformations 394  
classification of tumors of kidney, 37  
epidermoid cyst, dermoid cyst and teratoma, 153

**Tumors—Continued**

- genetic analysis of induction of tumors by methylcholanthrene, 2 mutations arising in mice following injection of methylcholanthrene 232
- morphologic alterations of neuron due to tumor invasion, 221
- some endocrinologic considerations of canine neoplastic diseases, 162
- Typhus**, morbid anatomy as seen in recent Guatemalan epidemic, observations on disease, 226
- Ultraviolet Rays** See Radiations
- Uranyl nitrate**, experimental nephropathies, renal phosphatase after poisoning with mercury bichloride, uranyl nitrate and potassium dichromate, 133
- Urinary Tract** See Kidneys
- Uterus**, changes after eradication of endometrial adenocarcinoma by radiotherapy with reference to infarct-like radionecrotic plaque in lining 237
- effects of abortifacient paste ('*utra-jel*') report of death from its use and of experimental study of its effects on rabbits and rats 91

**Uterus—Continued**

- proliferation of muscle cells in myometrium of non-pregnant uterus 323
- Utra-jel** See Abortion
- Wallace J. L.** Two cases of congenital web of bronchus, 47
- Weber-Christian Disease** See Panniculitis
- Weber-Sturge Disease** See Angioma
- Wilson, L. A. Jr.** Esophageal carcinoma in British West Indian and Panamanian Negroes, study of incidence etiologic factors and pathologic anatomy in 50 cases 79
- Wilson, S. I.** Mediastinal teratoma 113
- Wood, Francis Carter**, unveiling of portrait of 280
- Work**, local hydrostatic hypertension physical labor and static and gravitational factors, 56
- physical labor and hypercholesteremia 131
- Wounds** healing in skin of mice painted with 20-methylcholanthrene 257
- Xanthoma** hypercholesteremia and tubercous xanthomatosis 127
- Xanthomatosis** See Xanthoma

